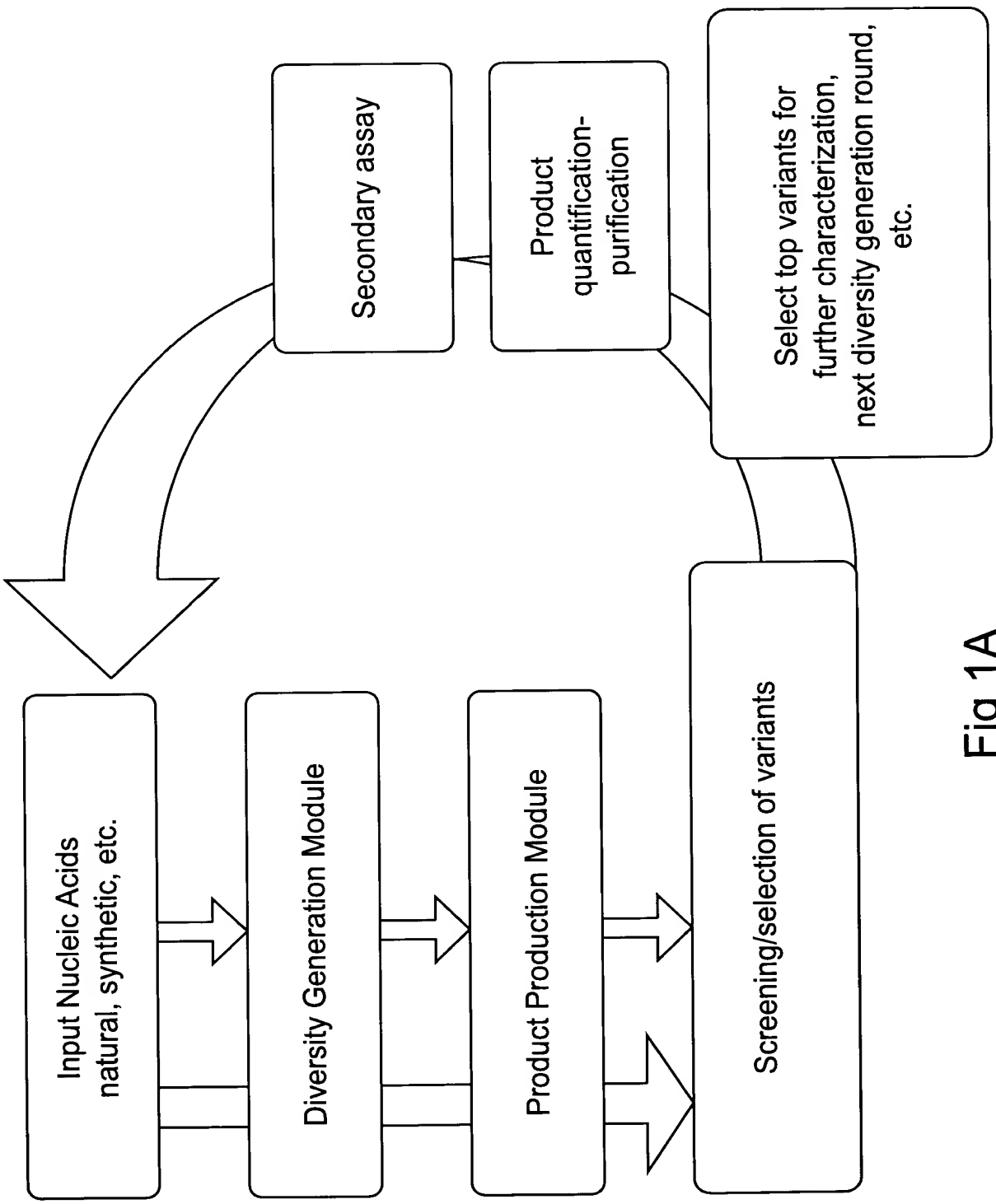

Input Nucleic Acids
natural, synthetic, etc.
Diversity Generation Module
Product Production Module
Screening/selection of variants
Secondary assay
Product quantification-purification
Select top variants for further characterization, next diversity generation round, etc.

Fig 1A

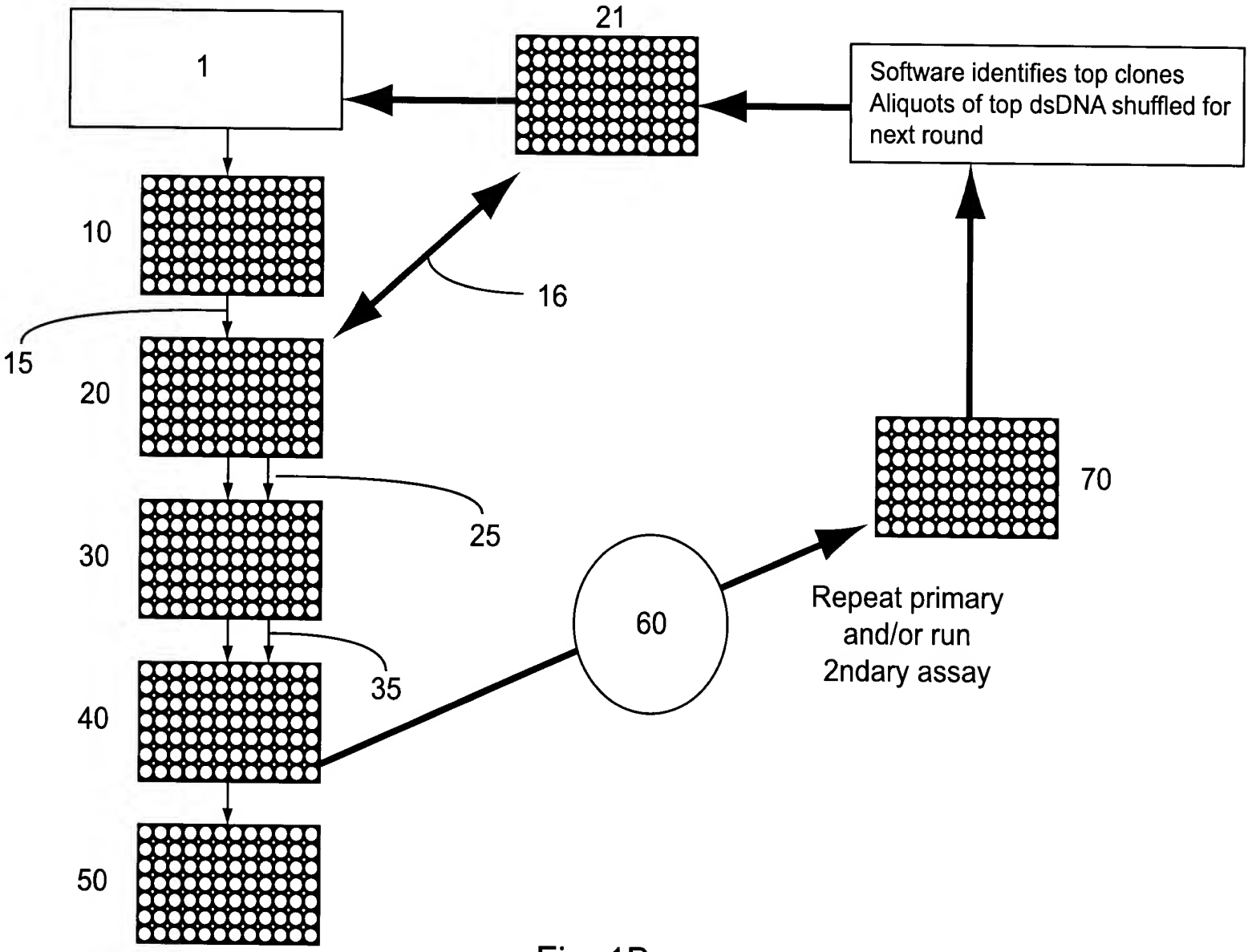

1
21
Software identifies top clones
Aliquots of top dsDNA shuffled for
next round
10
16
15
20
25
30
70
60
Repeat primary
and/or run
2ndary assay
35
40
50

Fig. 1B

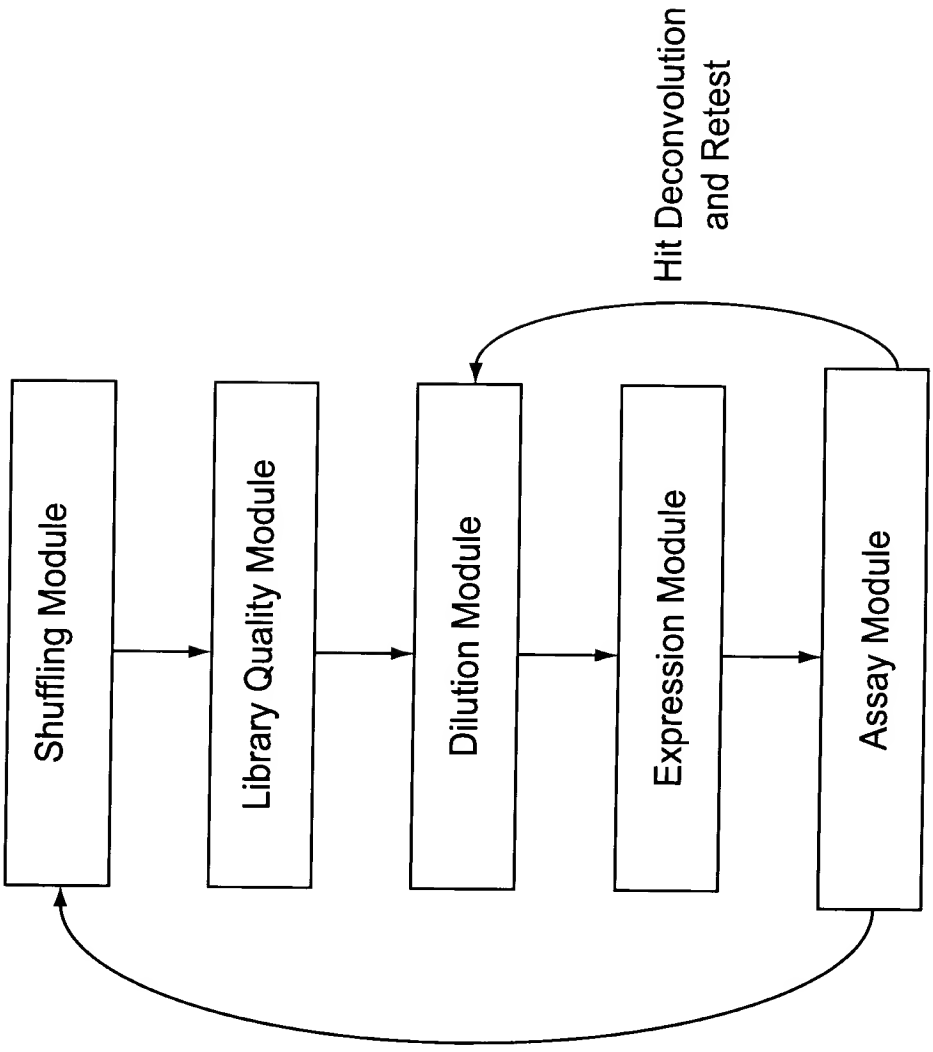


Fig. 2

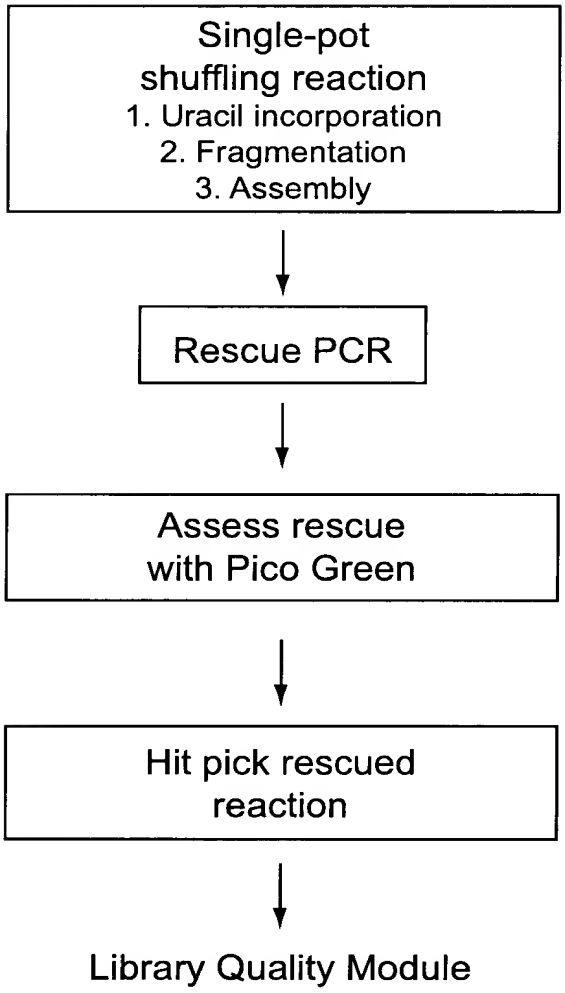

Single-pot
shuffling reaction
1. Uracil incorporation
2. Fragmentation
3. Assembly
Rescue PCR
Assess rescue
with Pico Green
Hit pick rescued
reaction
Library Quality Module

Fig. 3

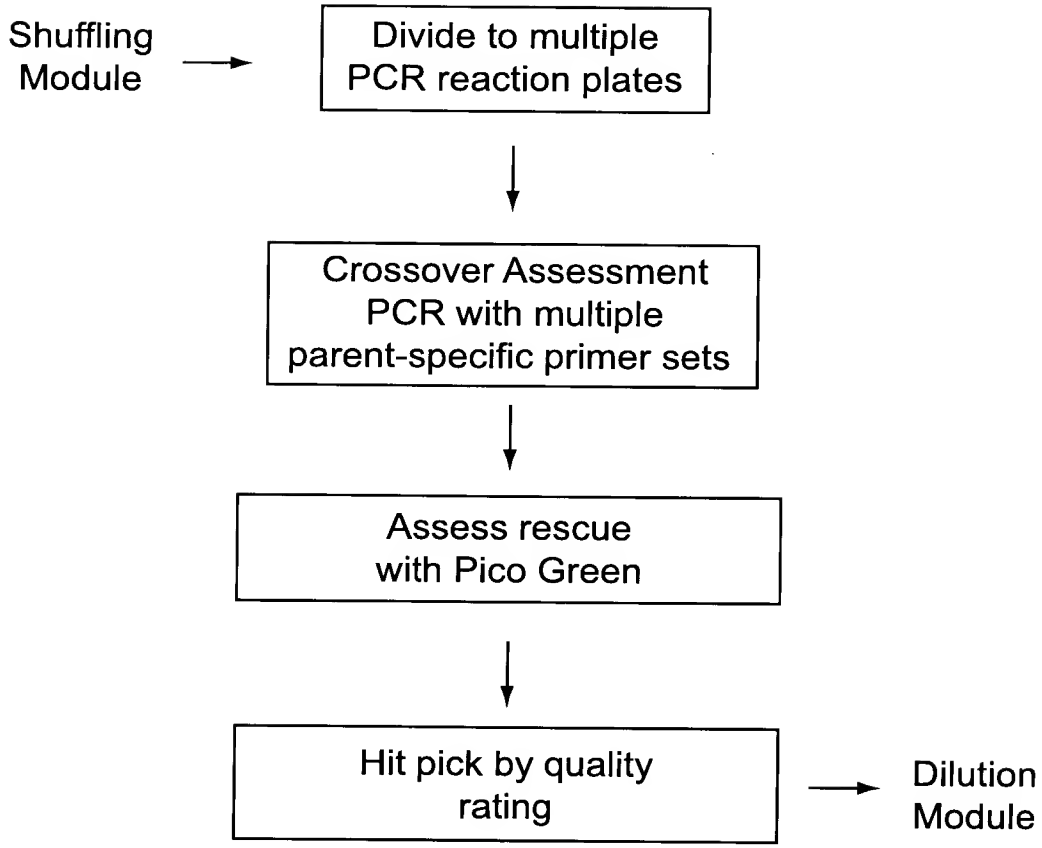

Shuffling Module
Divide to multiple PCR reaction plates
Crossover Assessment PCR with multiple parent-specific primer sets
Assess rescue with Pico Green
Hit pick by quality rating
Dilution Module

Fig. 4

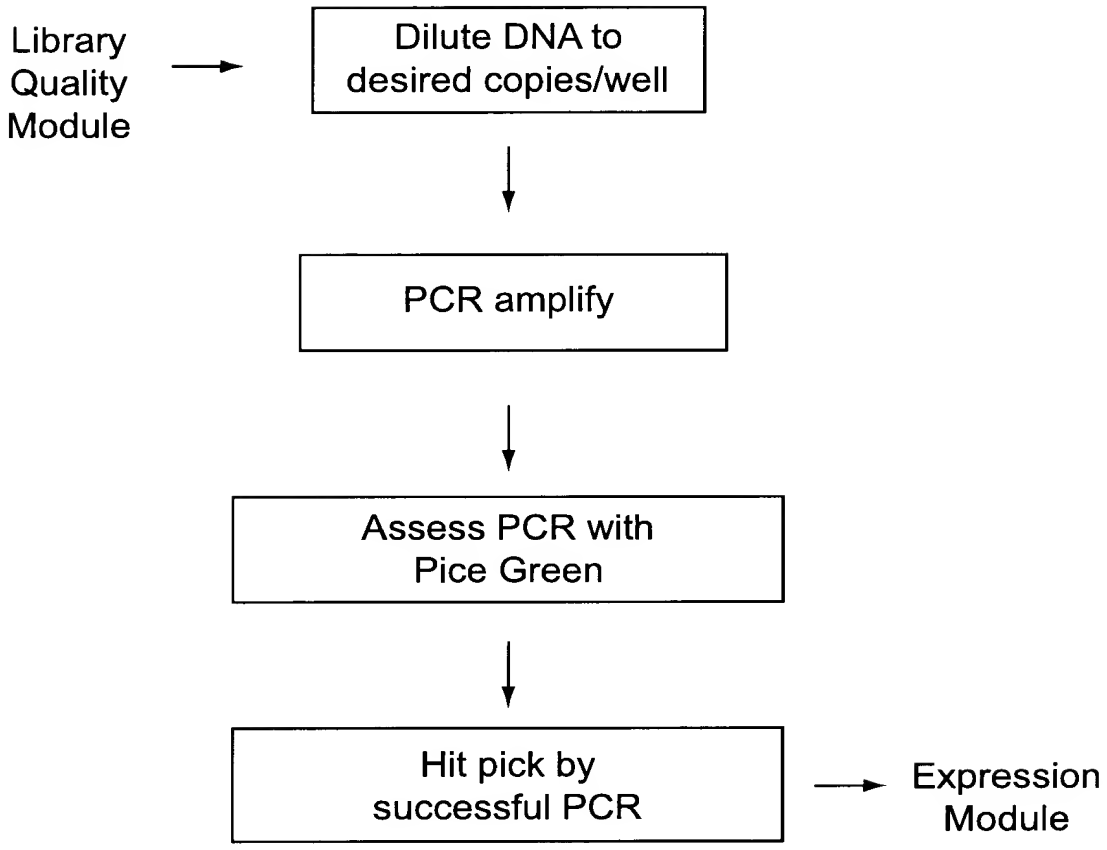


Fig. 5

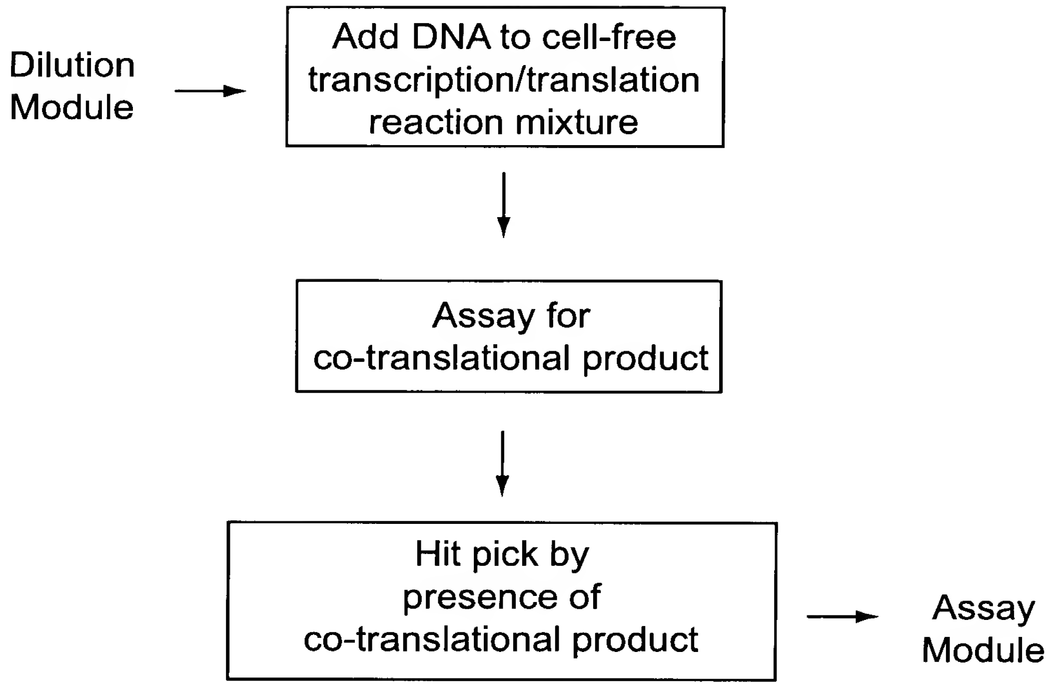

Dilution Module
Add DNA to cell-free transcription/translation reaction mixture
Assay for co-translational product
Hit pick by presence of co-translational product
Assay Module

Fig. 6

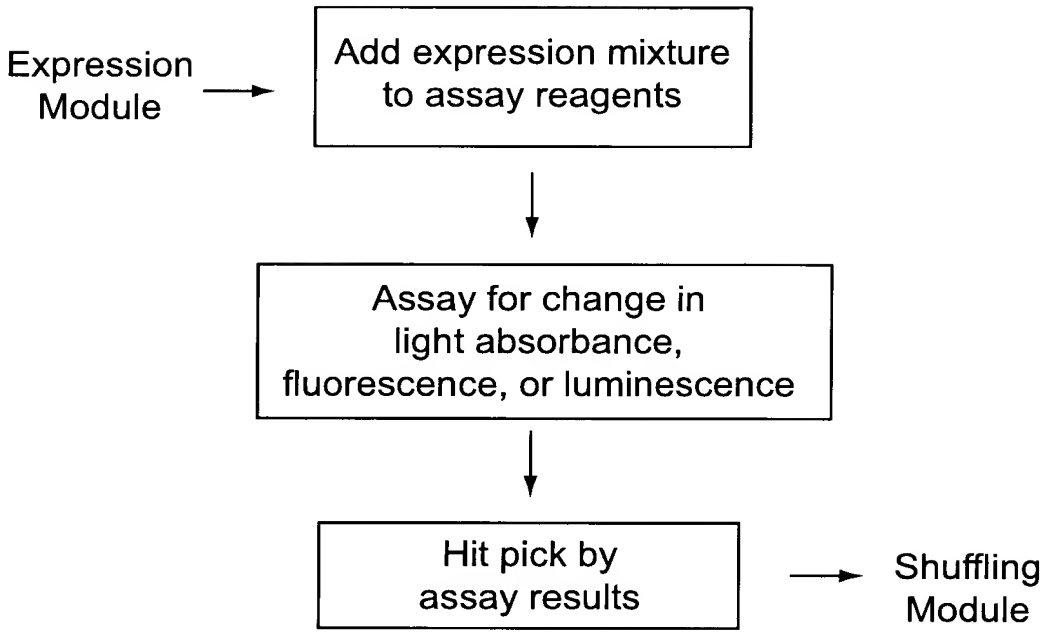

Expression Module
Add expression mixture to assay reagents
Assay for change in light absorbance, fluorescence, or luminescence
Hit pick by assay results
Shuffling Module

Fig. 7

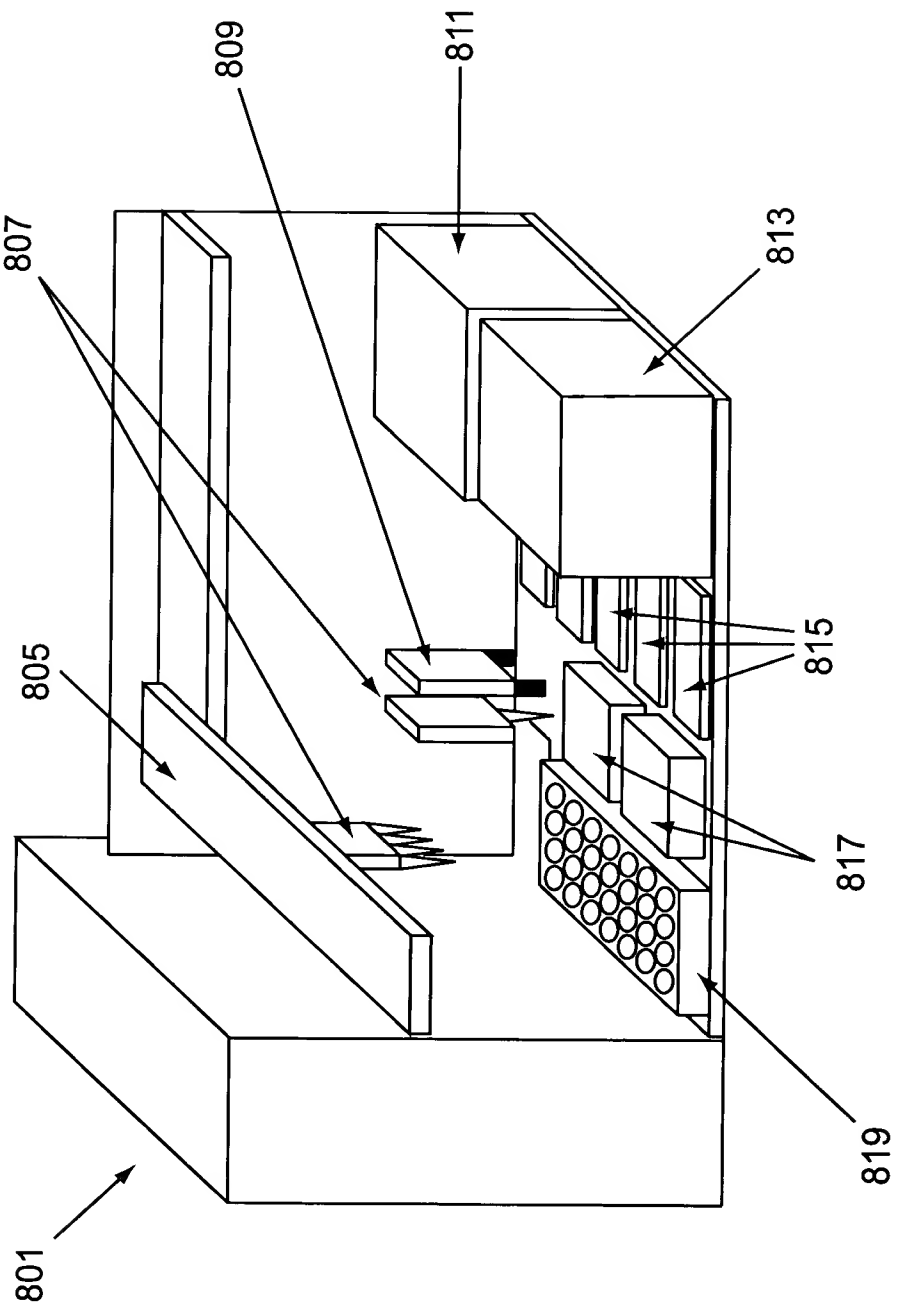


Fig. 8

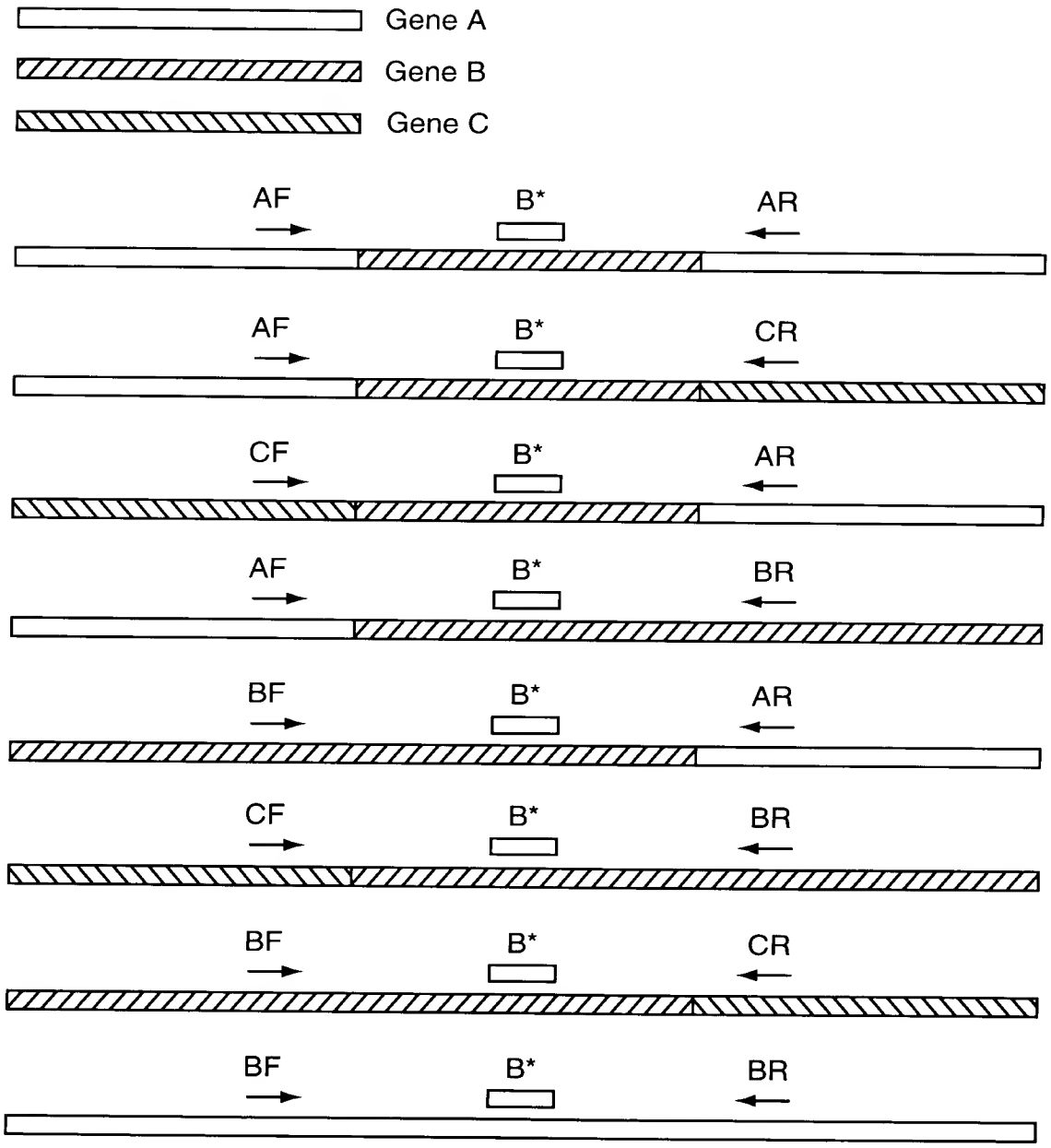
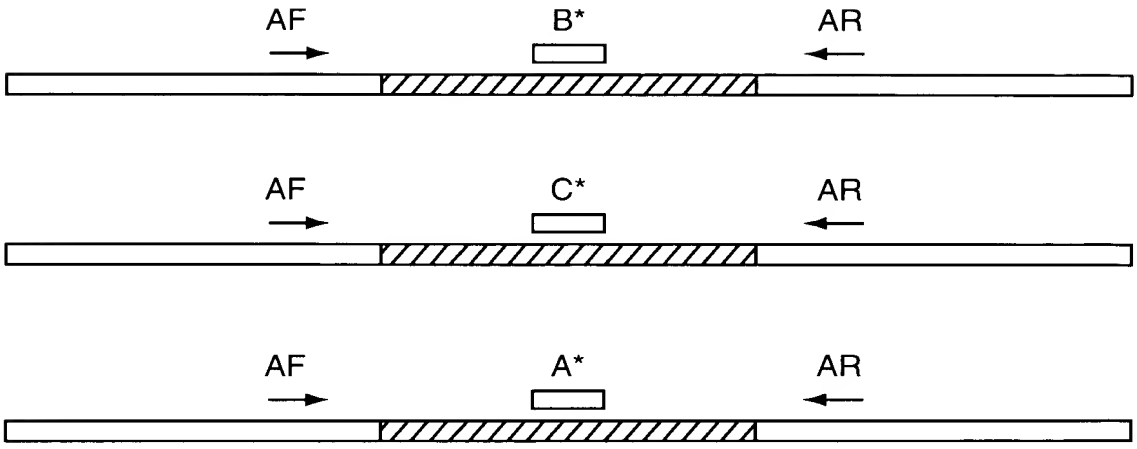

Gene A
Gene B
Gene C
AF
B*
AR
AF
B*
CR
CF
B*
AR
AF
B*
BR
BF
B*
AR
CF
B*
BR
BF
B*
CR
BF
B*
BR

Fig. 9A



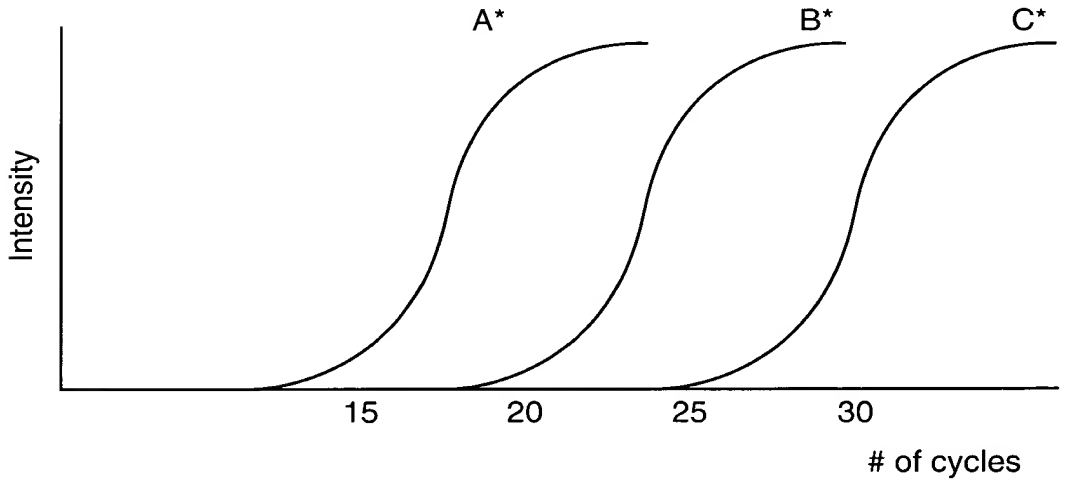


Fig. 9B

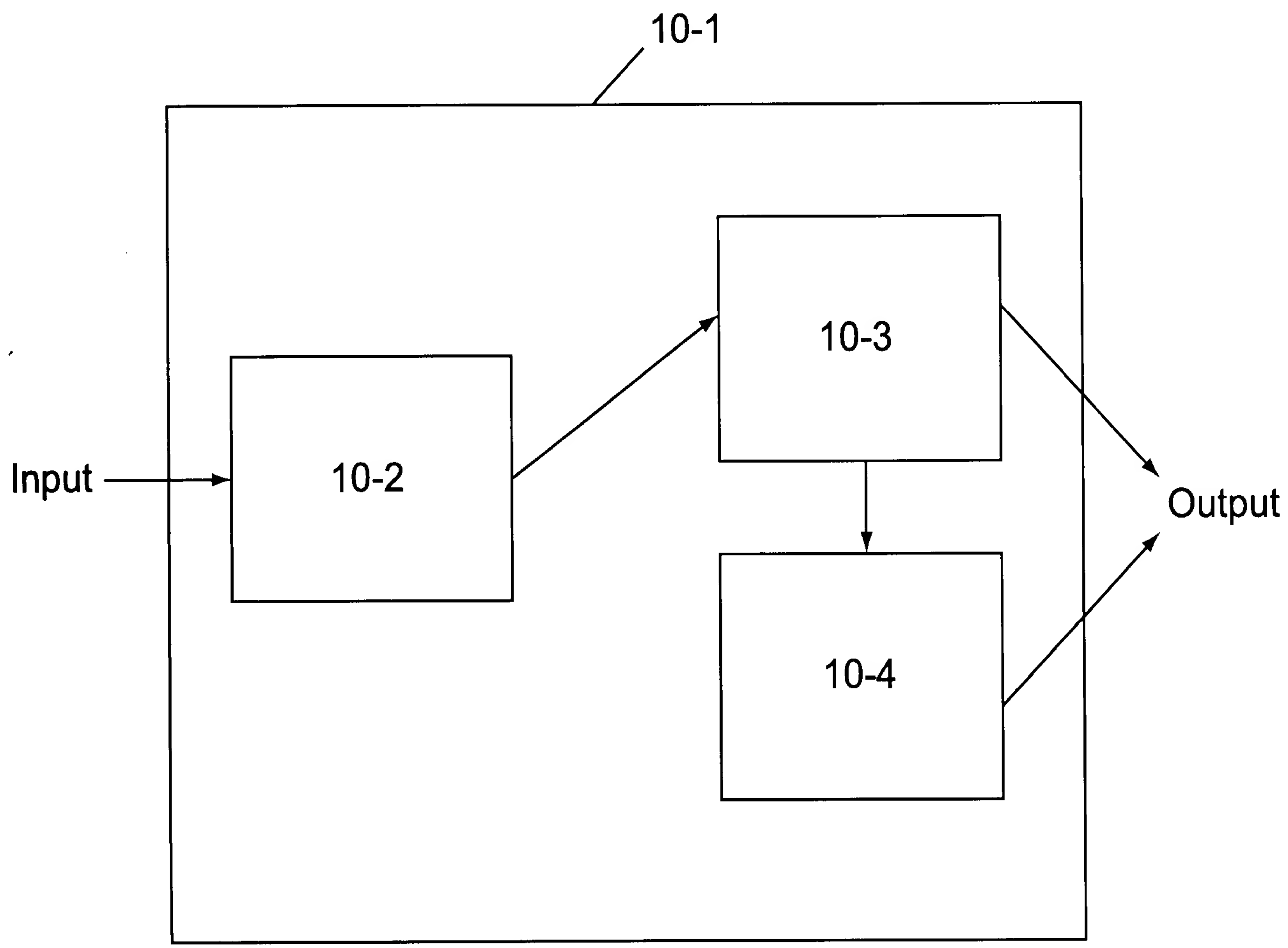


Fig. 10

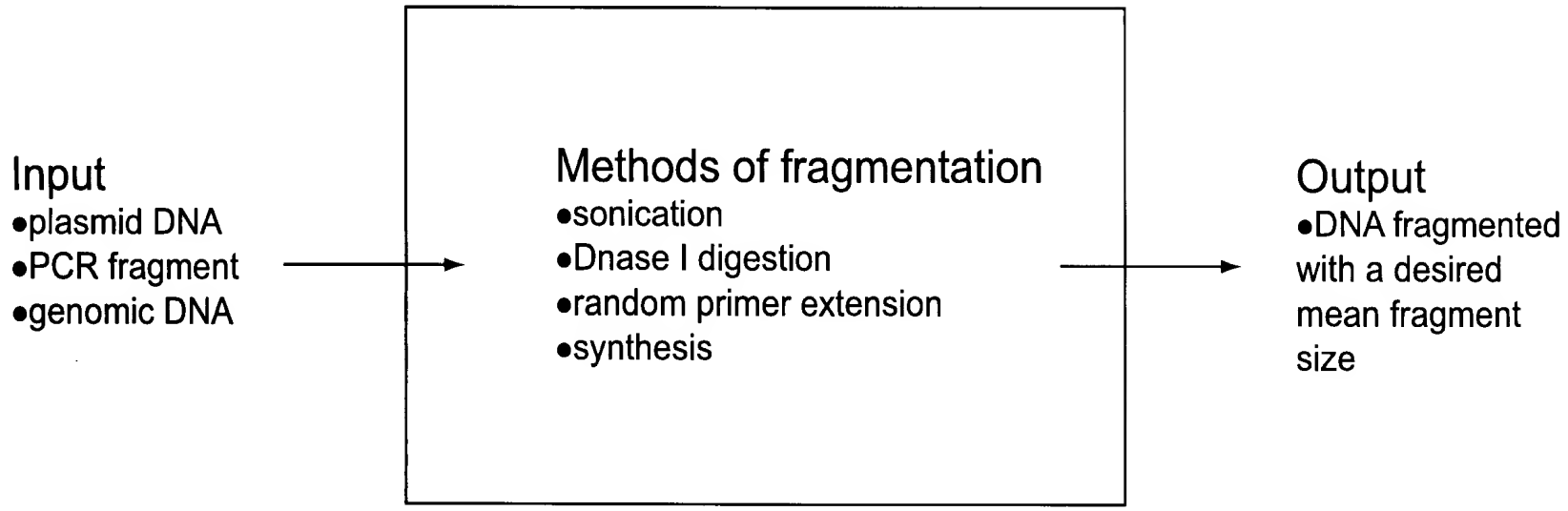

Input
•plasmid DNA
•PCR fragment
•genomic DNA
Methods of fragmentation
•sonication
•Dnase I digestion
•random primer extension
•synthesis
Output
•DNA fragmented with a desired mean fragment size

Fig. 11

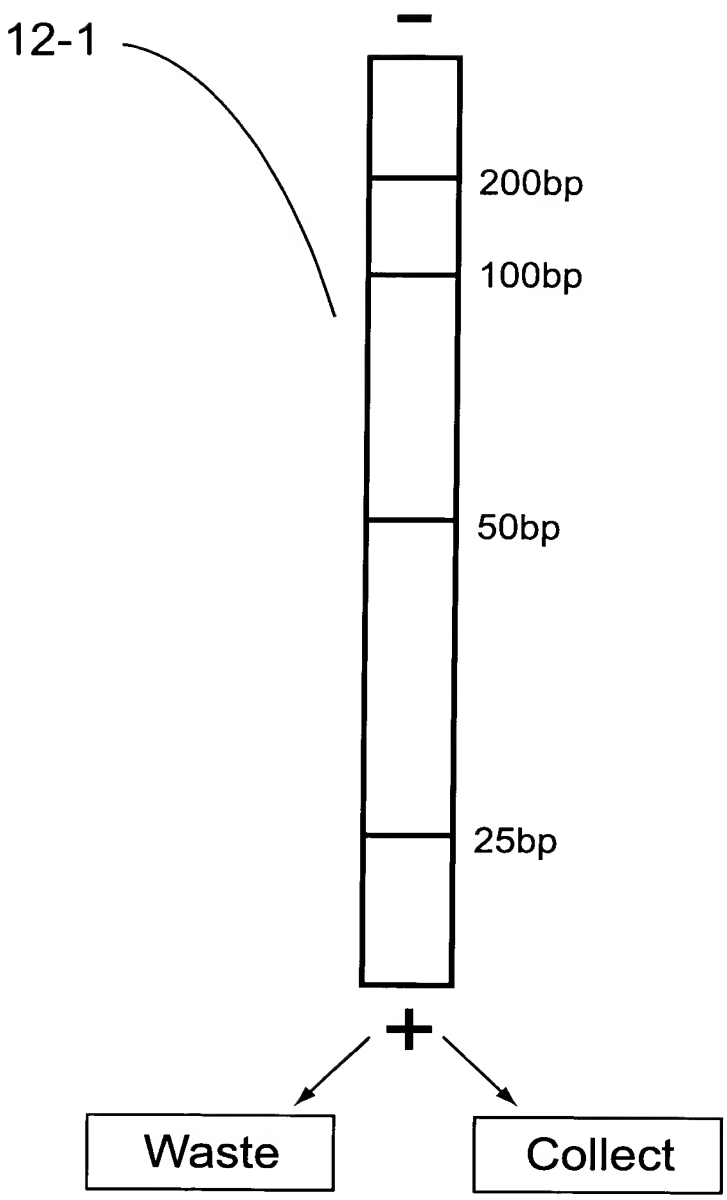


Fig. 12

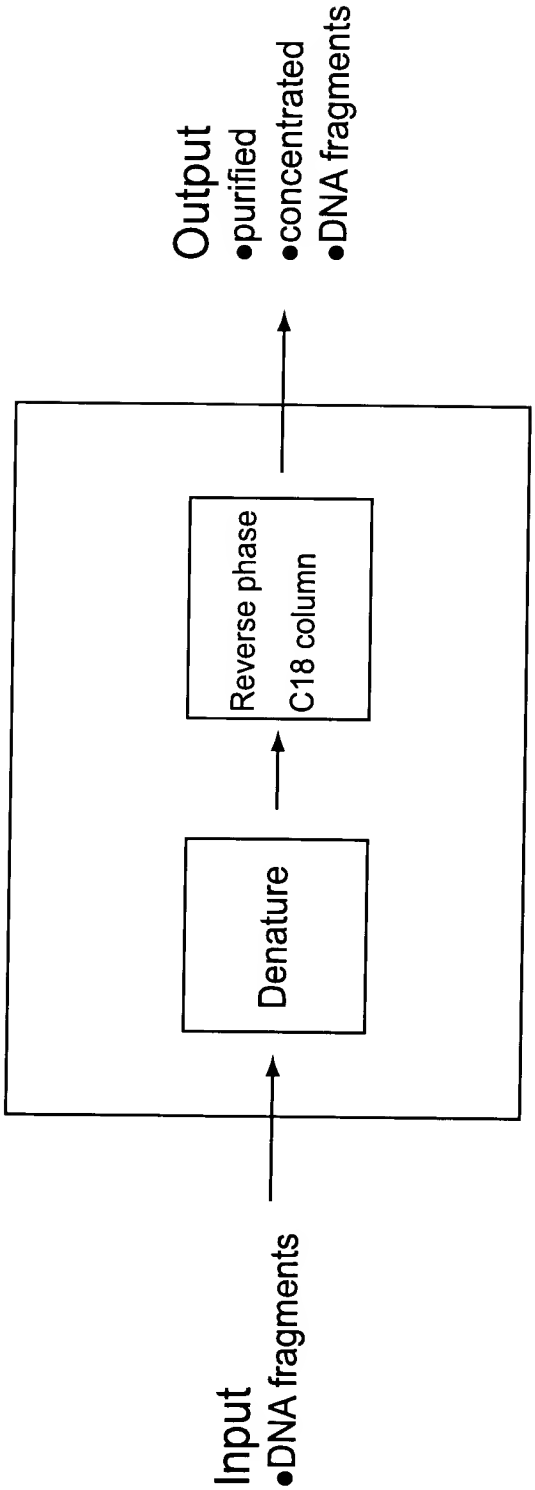

Input
•DNA fragments
Denature
Reverse phase
C18 column
Output
•purified
•concentrated
•DNA fragments

Fig. 13

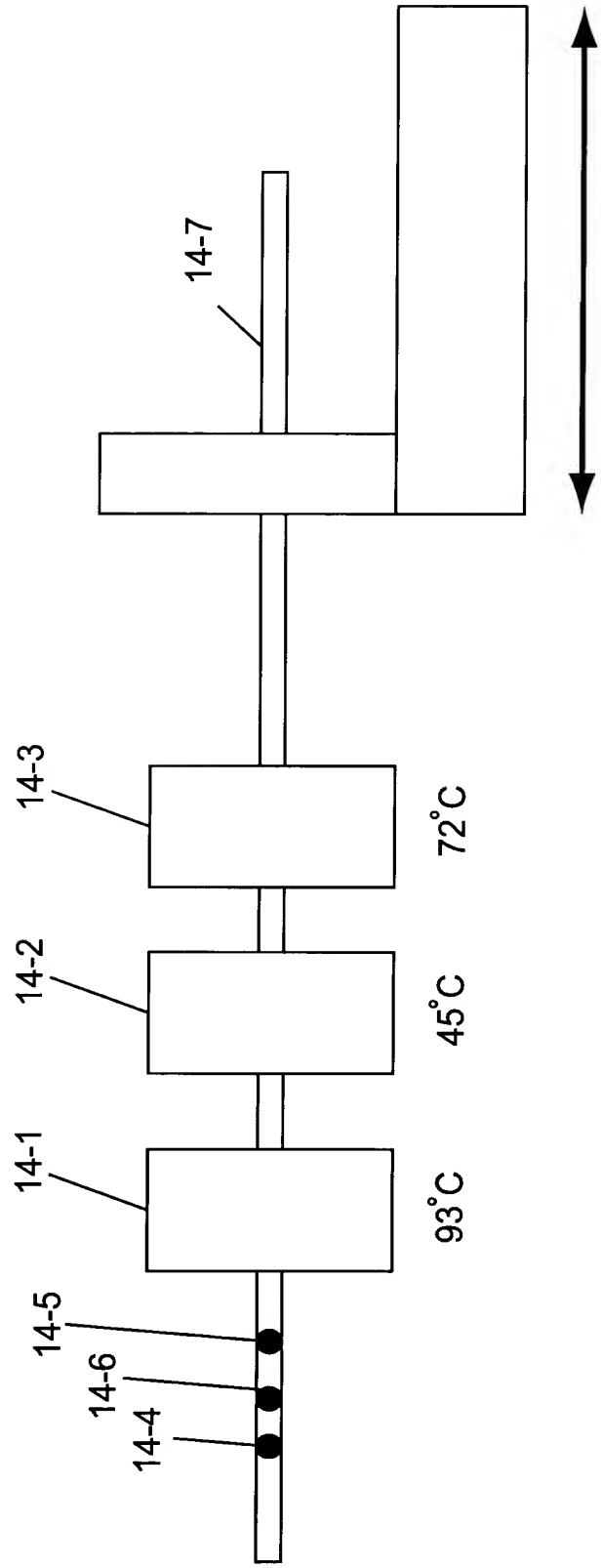


Fig. 14

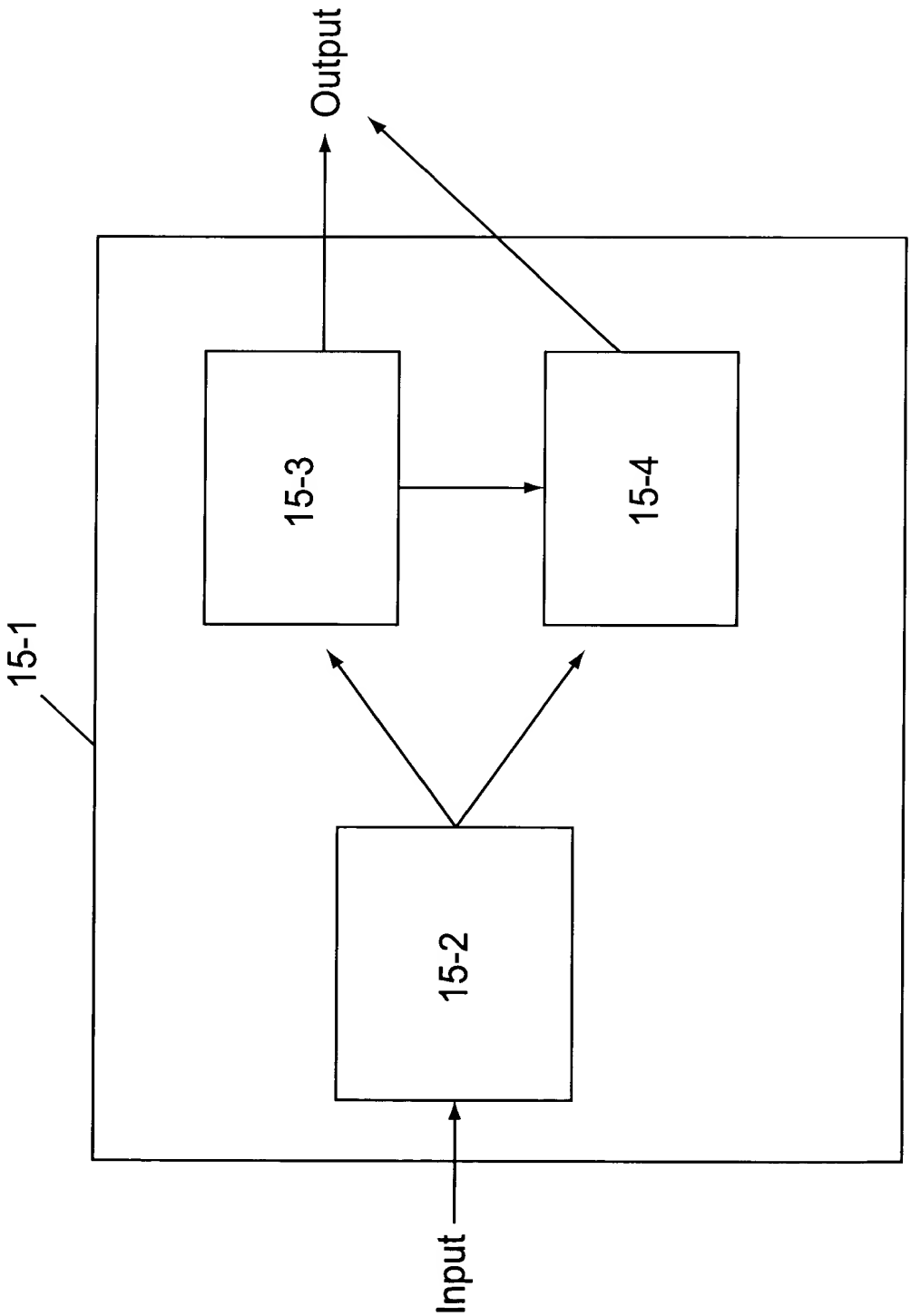


Fig. 15

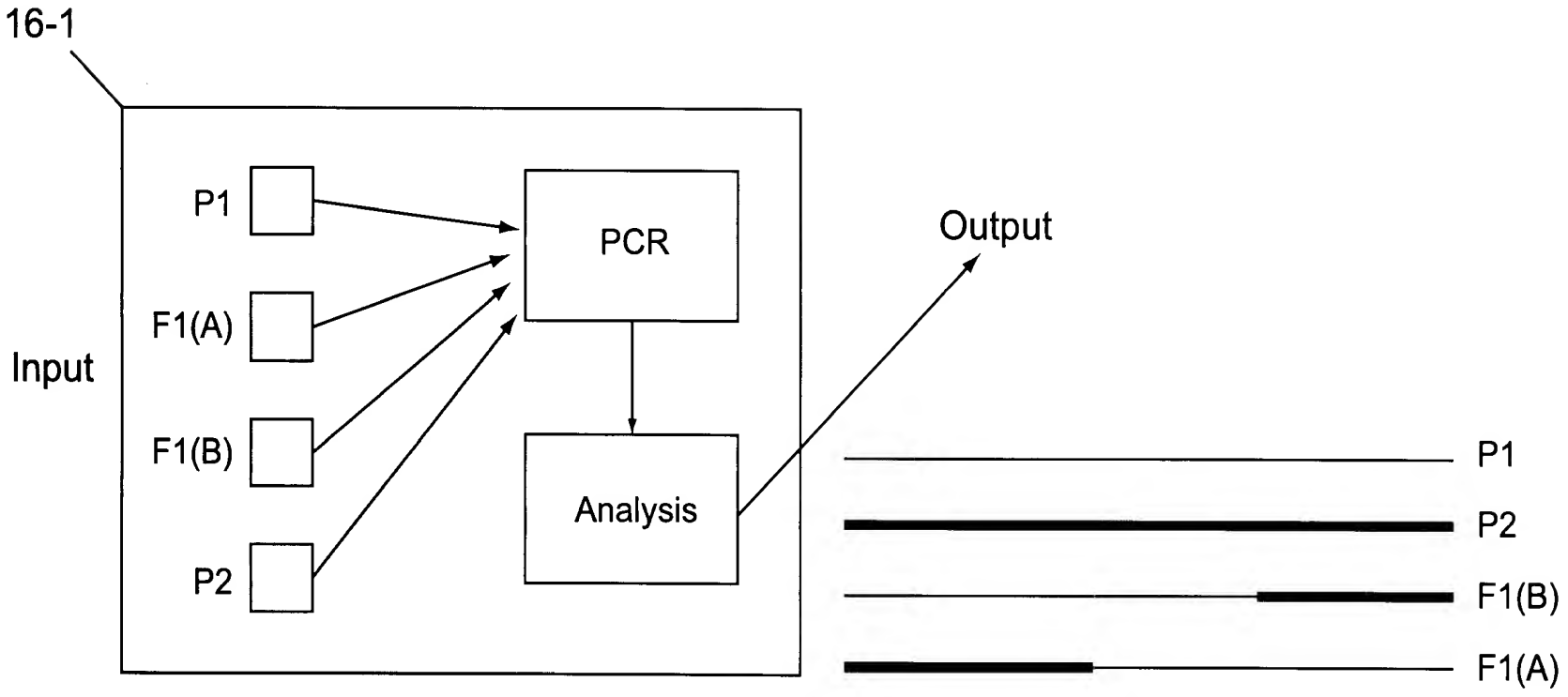


Fig. 16

Enrichment method for ivTT products

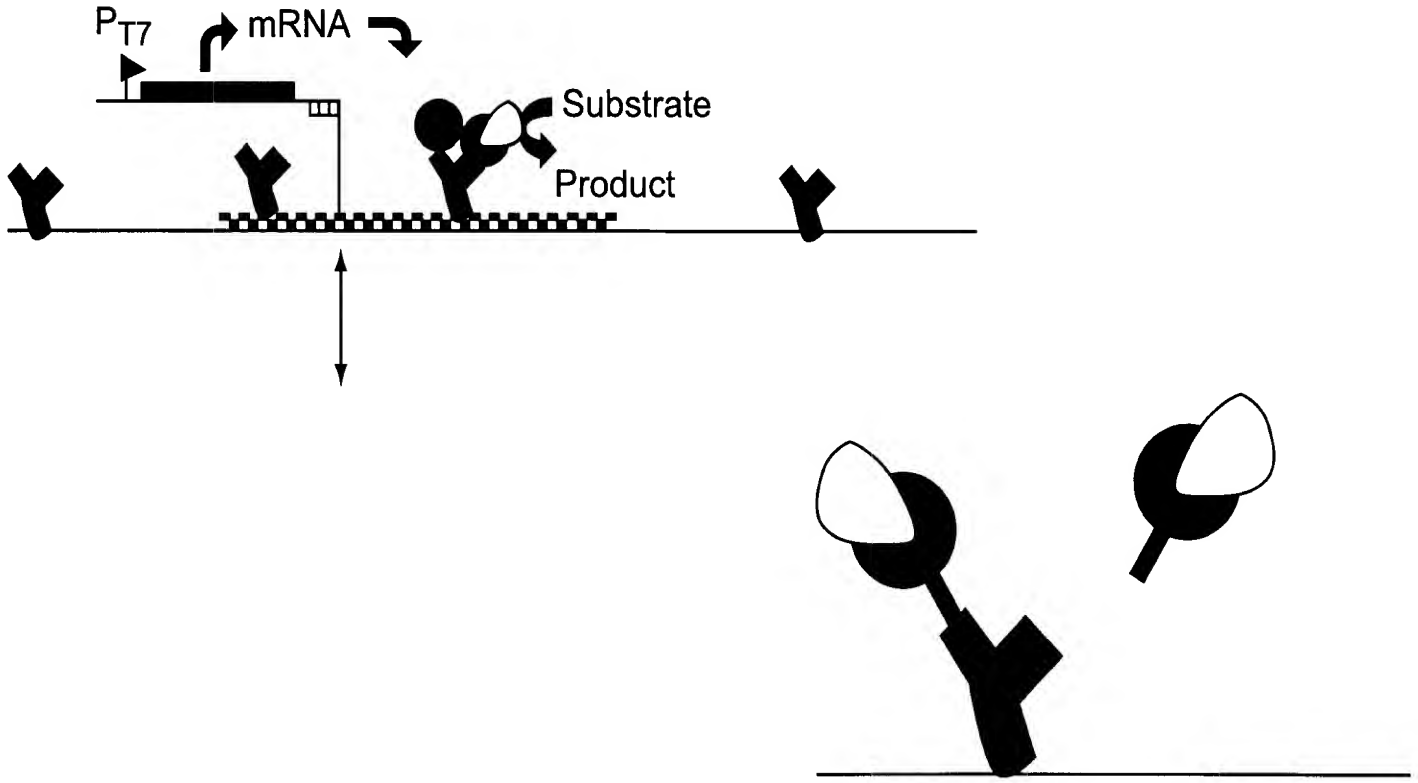


Fig. 17A

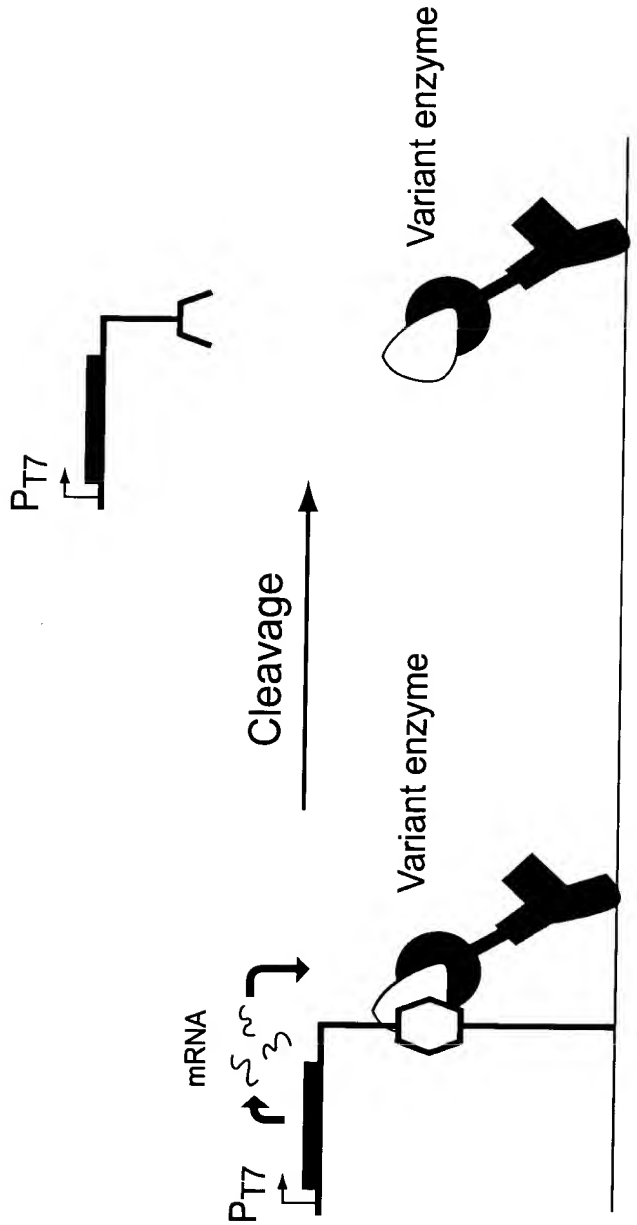


Fig. 17B

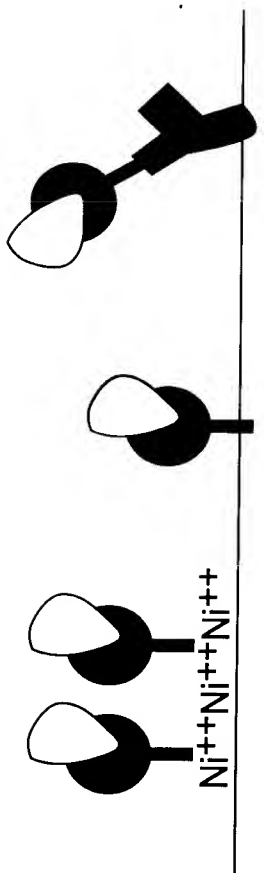


Fig. 17C

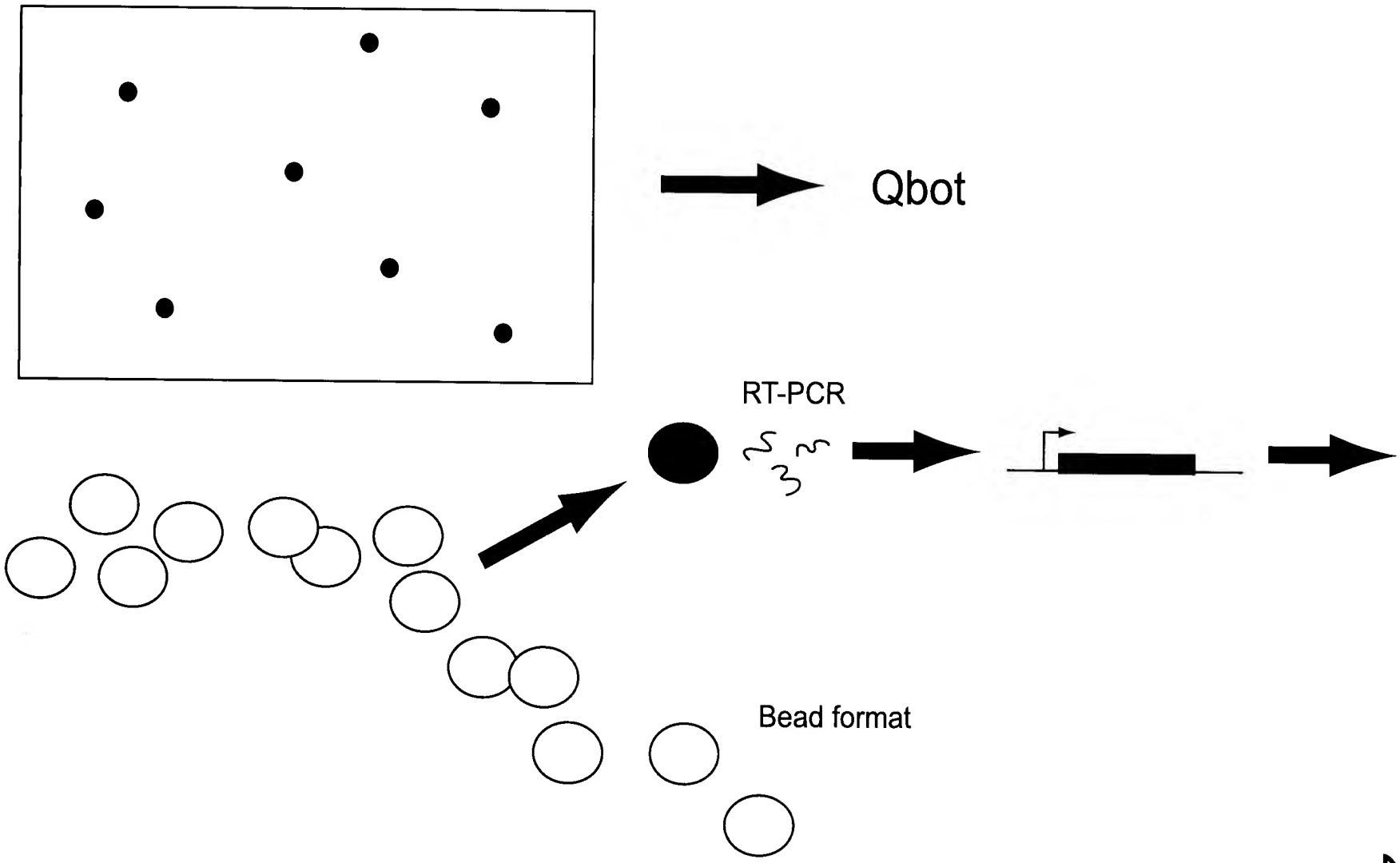


Fig. 17D

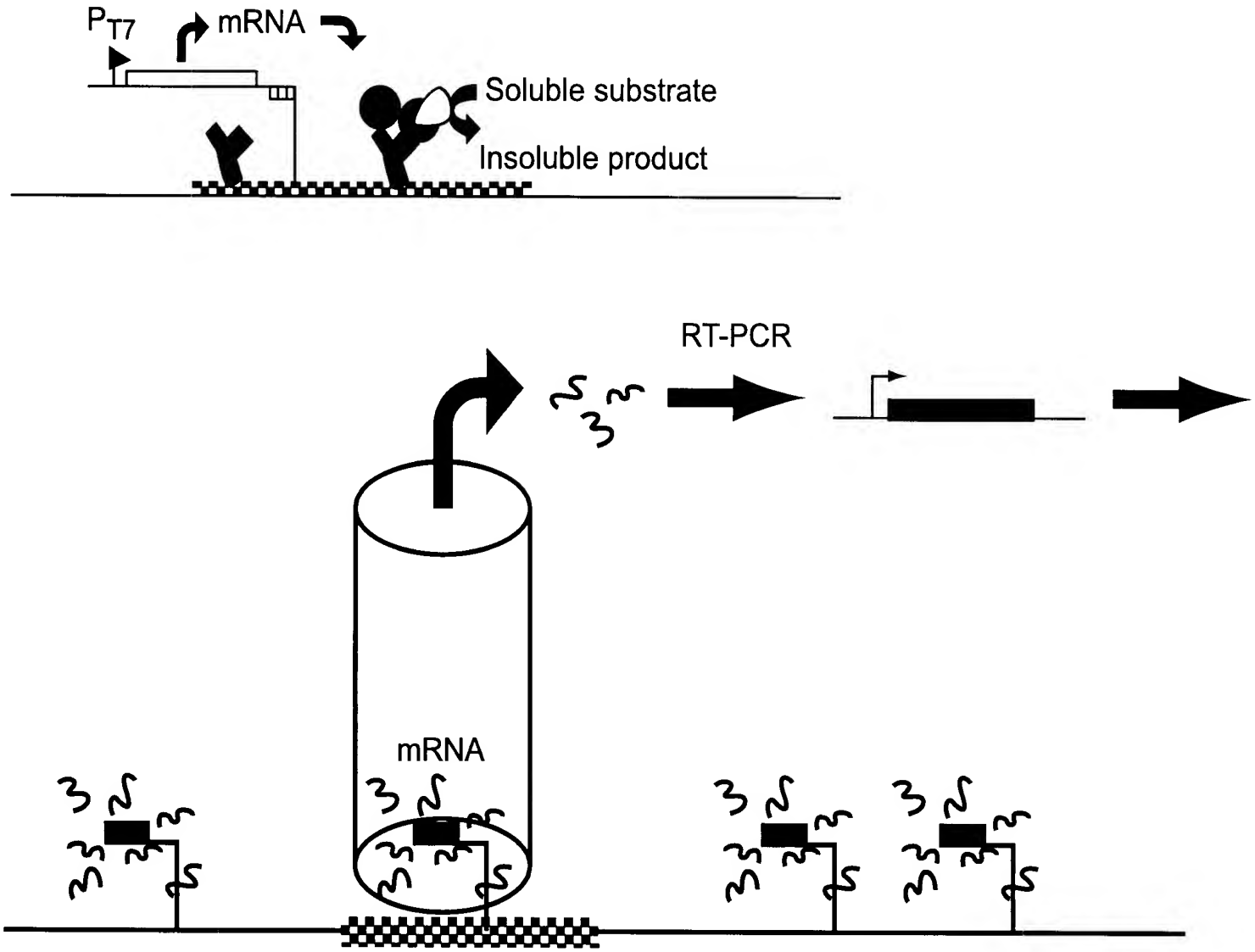


Fig. 17E

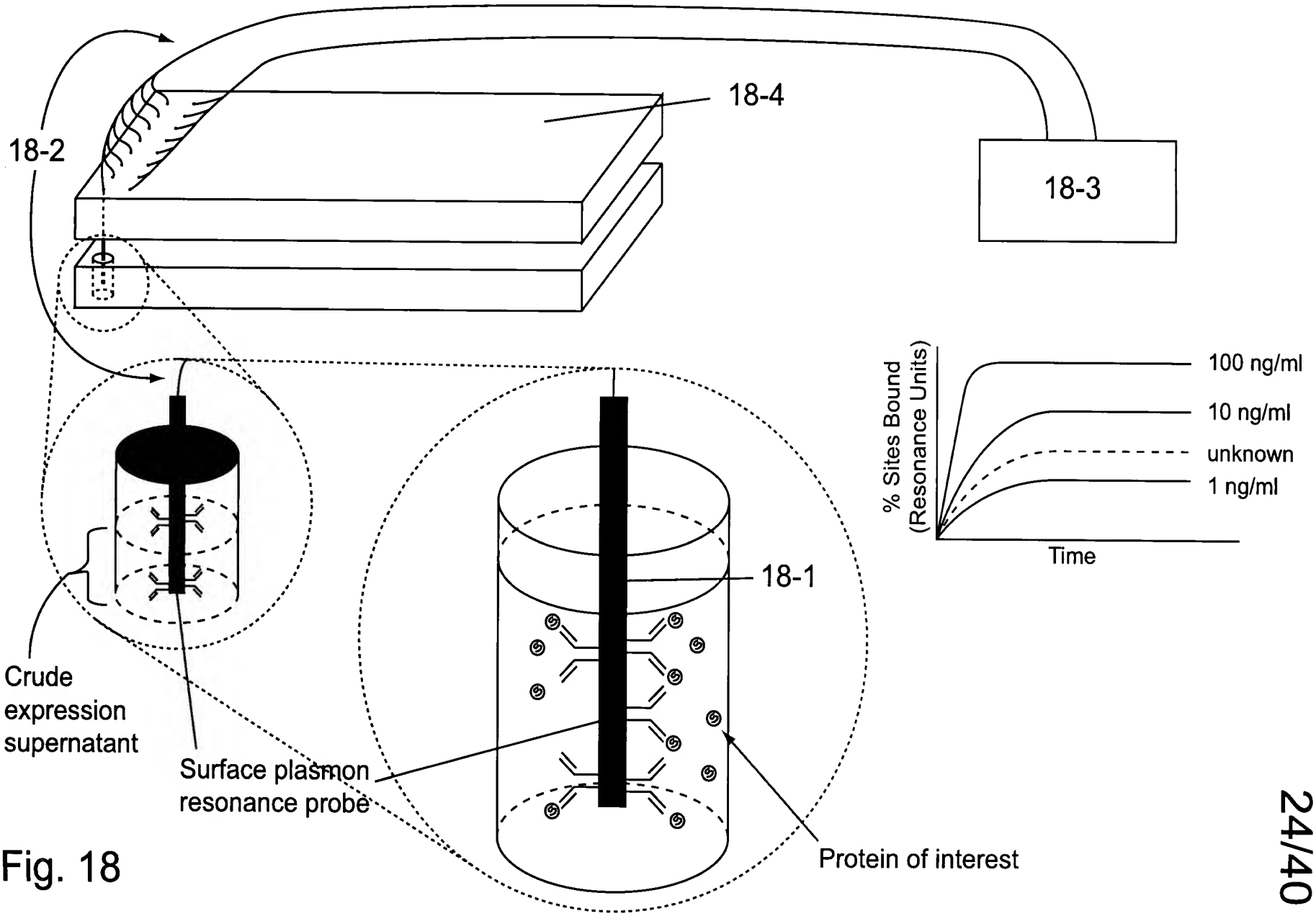


Fig. 18

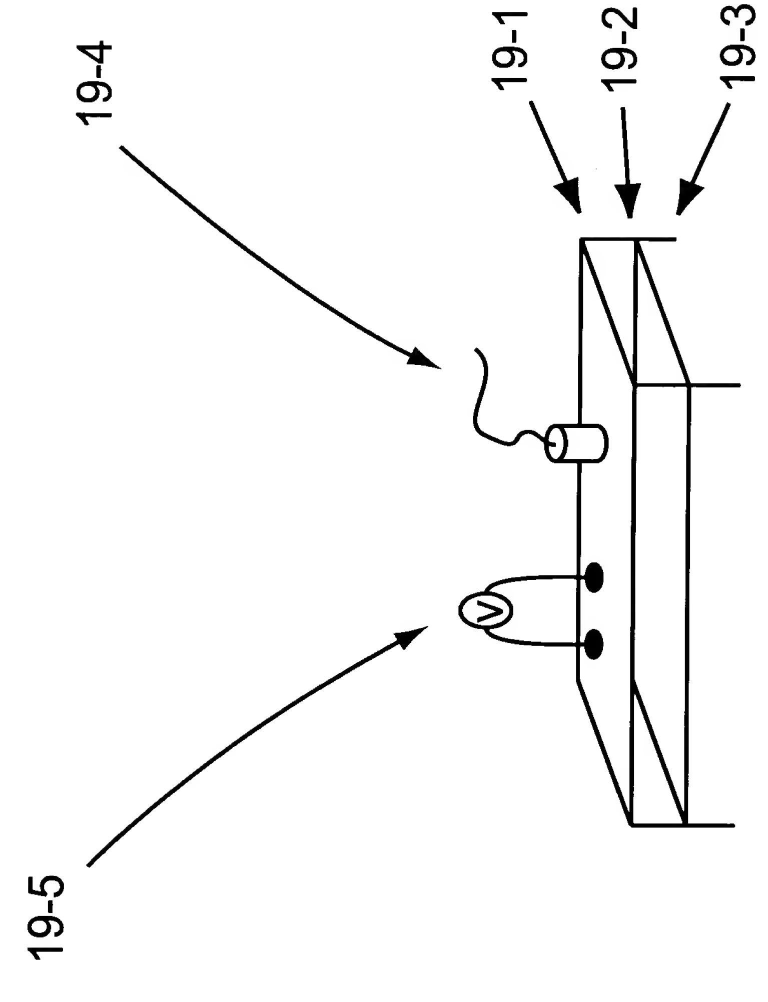


Fig. 19

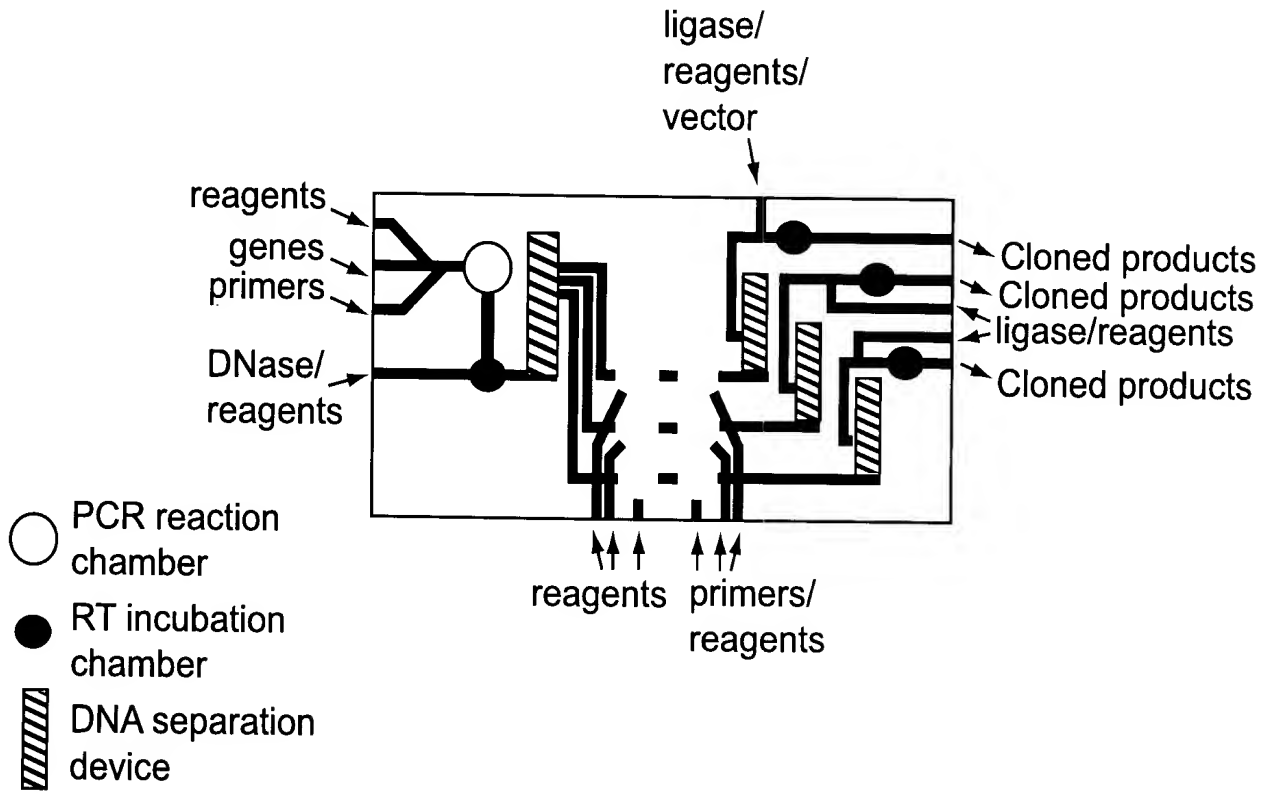
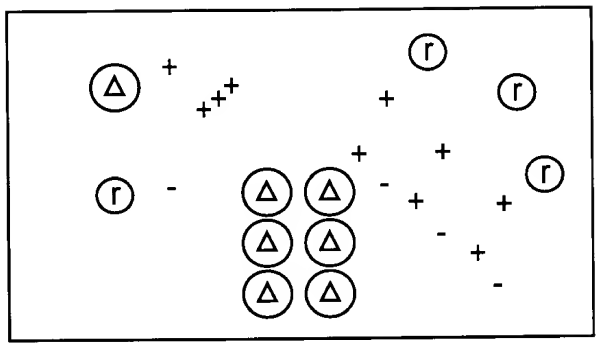


Fig. 20



temperature controller

incubator

DNA separator

DNA separator, incl. fragment pruifier

Fig. 21

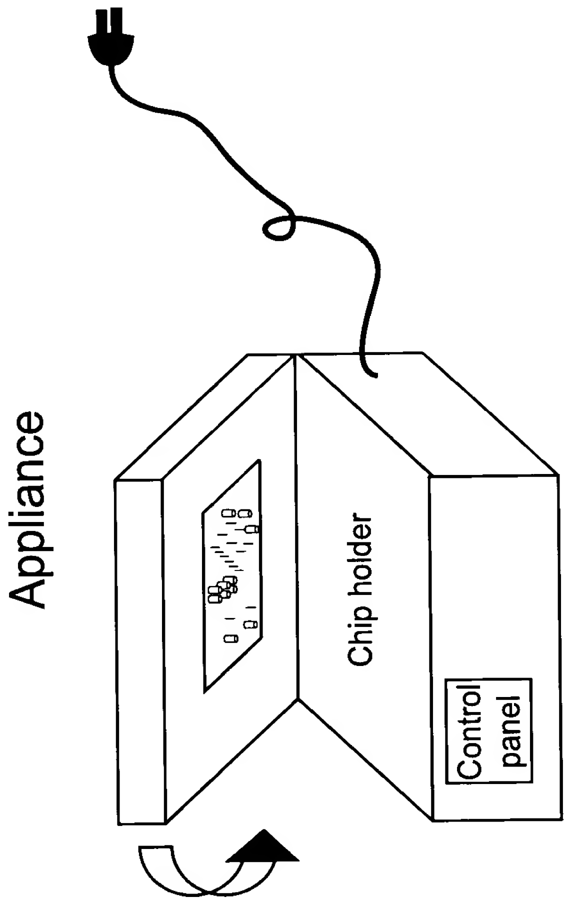


Fig. 22

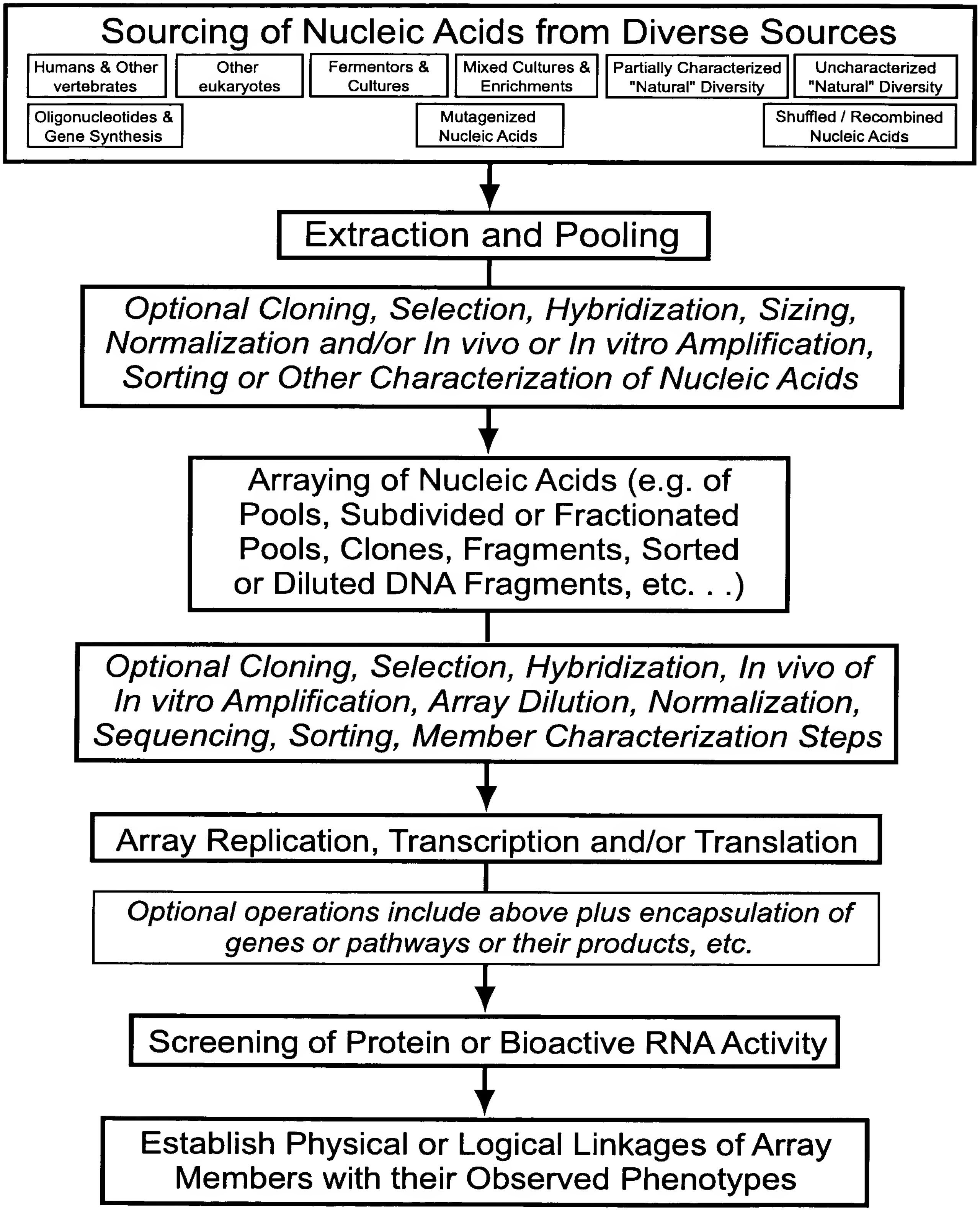

Sourcing of Nucleic Acids from Diverse Sources
Humans & Other vertebrates
Other eukaryotes
Fermentors & Cultures
Mixed Cultures & Enrichments
Partially Characterized "Natural" Diversity
Uncharacterized "Natural" Diversity
Oligonucleotides & Gene Synthesis
Mutagenized Nucleic Acids
Shuffled / Recombined Nucleic Acids
Extraction and Pooling
Optional Cloning, Selection, Hybridization, Sizing, Normalization and/or In vivo or In vitro Amplification, Sorting or Other Characterization of Nucleic Acids
Arraying of Nucleic Acids (e.g. of Pools, Subdivided or Fractionated Pools, Clones, Fragments, Sorted or Diluted DNA Fragments, etc. . .)
Optional Cloning, Selection, Hybridization, In vivo of In vitro Amplification, Array Dilution, Normalization, Sequencing, Sorting, Member Characterization Steps
Array Replication, Transcription and/or Translation
Optional operations include above plus encapsulation of genes or pathways or their products, etc.
Screening of Protein or Bioactive RNA Activity
Establish Physical or Logical Linkages of Array Members with their Observed Phenotypes

Fig. 23

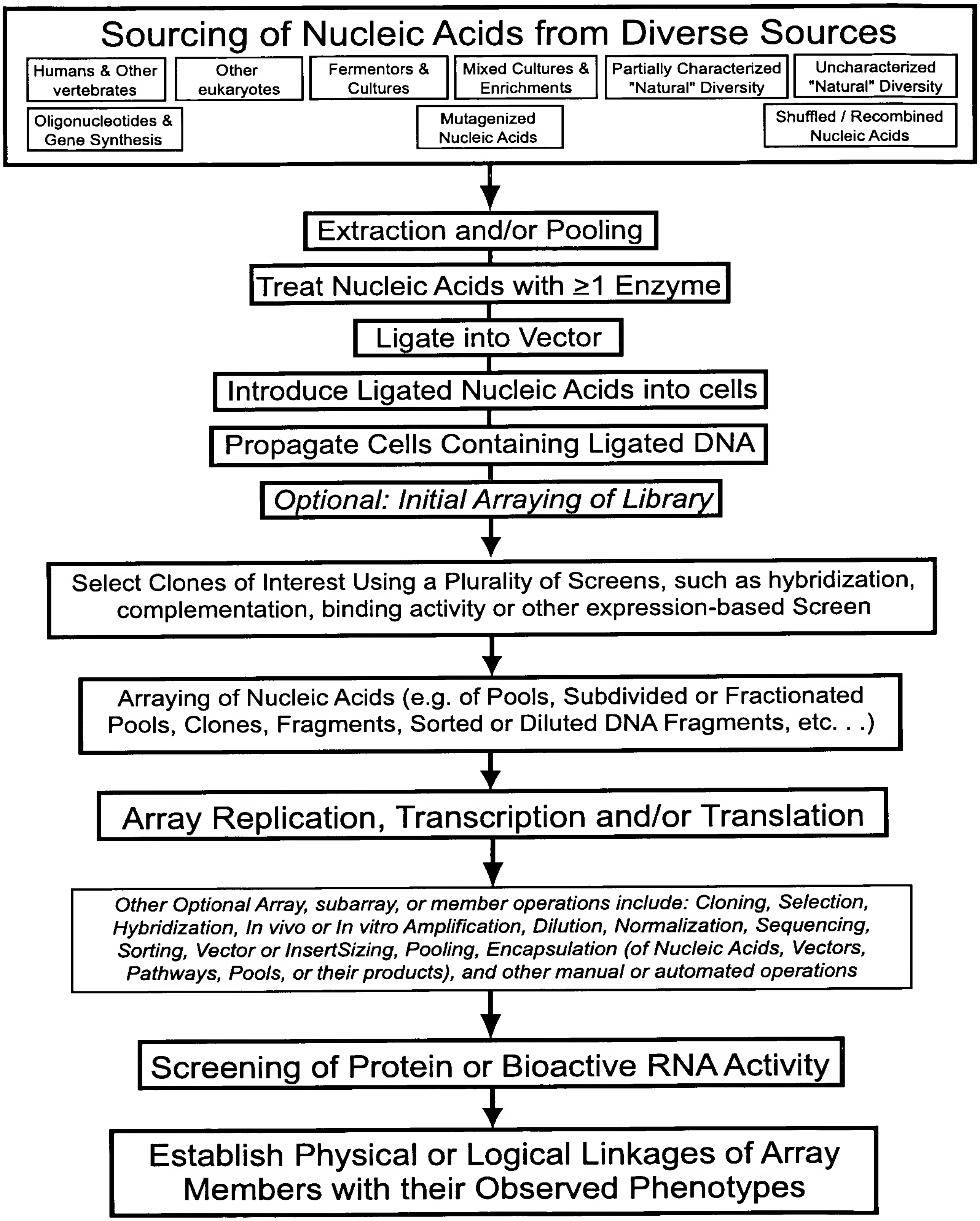
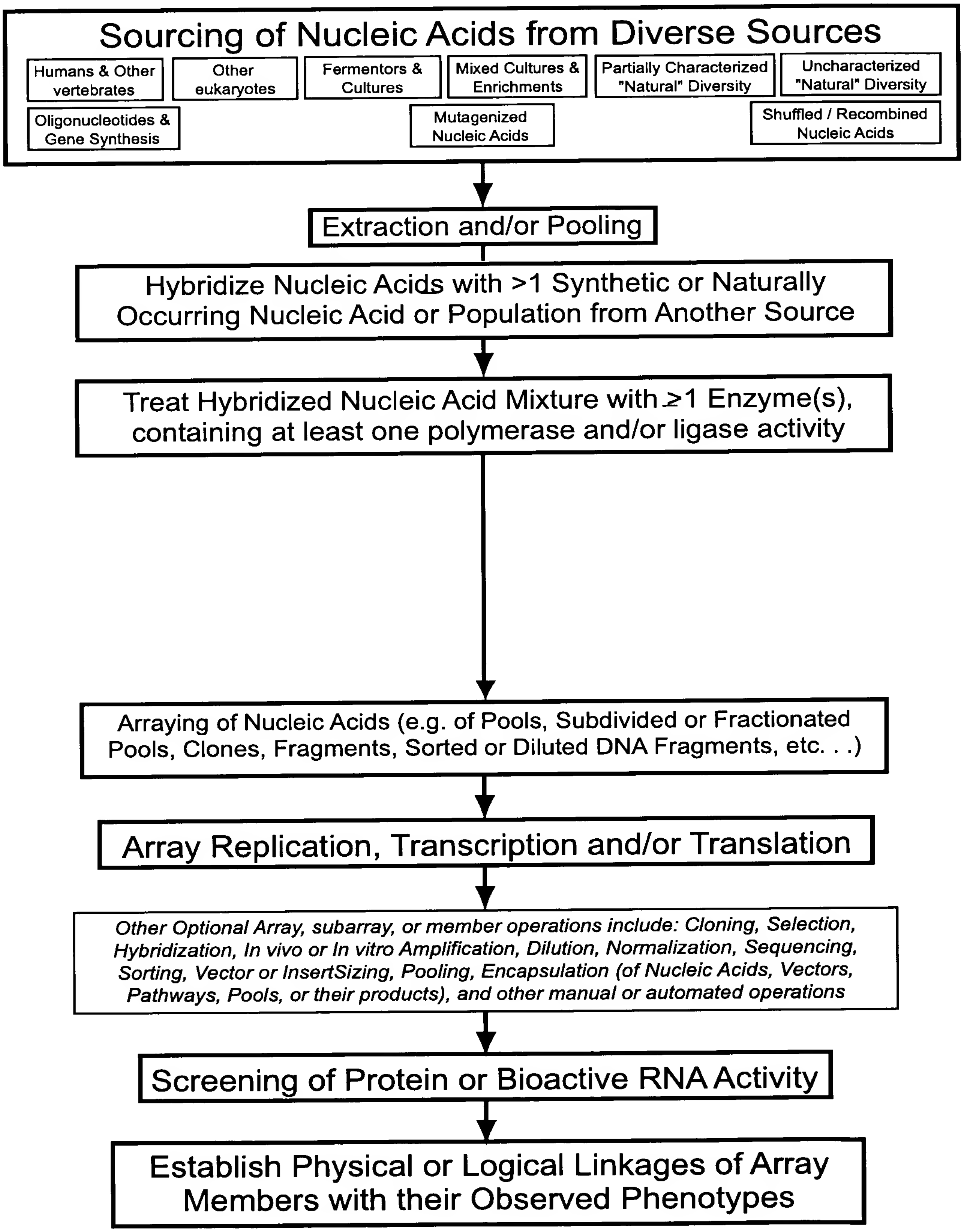

Sourcing of Nucleic Acids from Diverse Sources
Humans & Other vertebrates
Other eukaryotes
Fermentors & Cultures
Mixed Cultures & Enrichments
Partially Characterized "Natural" Diversity
Uncharacterized "Natural" Diversity
Oligonucleotides & Gene Synthesis
Mutagenized Nucleic Acids
Shuffled / Recombined Nucleic Acids
Extraction and/or Pooling
Treat Nucleic Acids with ≥1 Enzyme
Ligate into Vector
Introduce Ligated Nucleic Acids into cells
Propagate Cells Containing Ligated DNA
Optional: Initial Arraying of Library
Select Clones of Interest Using a Plurality of Screens, such as hybridization, complementation, binding activity or other expression-based Screen
Arraying of Nucleic Acids (e.g. of Pools, Subdivided or Fractionated Pools, Clones, Fragments, Sorted or Diluted DNA Fragments, etc. . .)
Array Replication, Transcription and/or Translation
Other Optional Array, subarray, or member operations include: Cloning, Selection, Hybridization, In vivo or In vitro Amplification, Dilution, Normalization, Sequencing, Sorting, Vector or InsertSizing, Pooling, Encapsulation (of Nucleic Acids, Vectors, Pathways, Pools, or their products), and other manual or automated operations
Screening of Protein or Bioactive RNA Activity
Establish Physical or Logical Linkages of Array Members with their Observed Phenotypes

Fig. 24


Sourcing of Nucleic Acids from Diverse Sources
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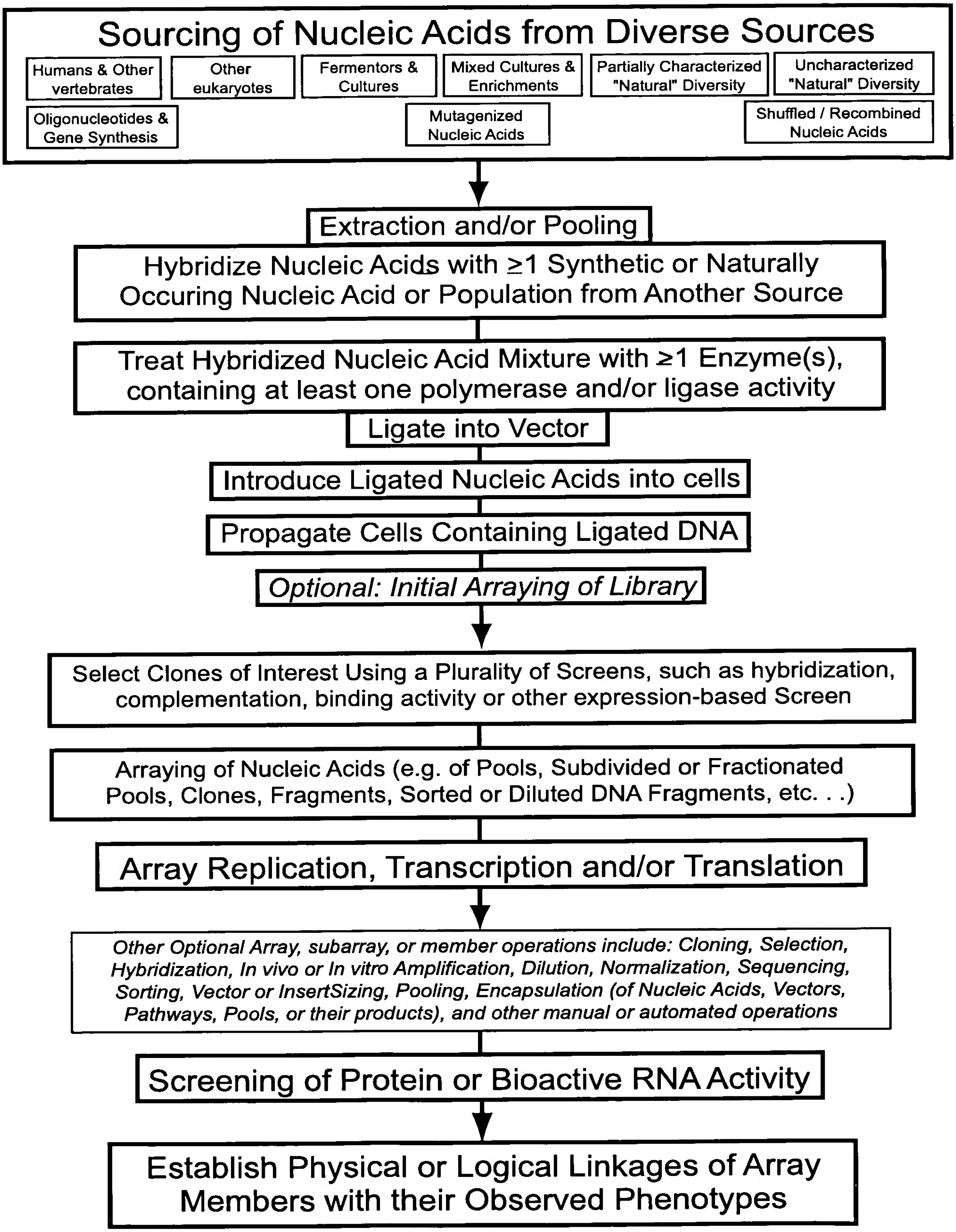
Mixed Cultures & Enrichments
Partially Characterized "Natural" Diversity
Uncharacterized "Natural" Diversity
Oligonucleotides & Gene Synthesis
Mutagenized Nucleic Acids
Shuffled / Recombined Nucleic Acids
Extraction and/or Pooling
Hybridize Nucleic Acids with >1 Synthetic or Naturally Occurring Nucleic Acid or Population from Another Source
Treat Hybridized Nucleic Acid Mixture with ≥1 Enzyme(s), containing at least one polymerase and/or ligase activity
Arraying of Nucleic Acids (e.g. of Pools, Subdivided or Fractionated Pools, Clones, Fragments, Sorted or Diluted DNA Fragments, etc. . .)
Array Replication, Transcription and/or Translation
Other Optional Array, subarray, or member operations include: Cloning, Selection, Hybridization, In vivo or In vitro Amplification, Dilution, Normalization, Sequencing, Sorting, Vector or InsertSizing, Pooling, Encapsulation (of Nucleic Acids, Vectors, Pathways, Pools, or their products), and other manual or automated operations
Screening of Protein or Bioactive RNA Activity
Establish Physical or Logical Linkages of Array Members with their Observed Phenotypes

Fig. 25


Sourcing of Nucleic Acids from Diverse Sources
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Uncharacterized "Natural" Diversity
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Extraction and/or Pooling
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Treat Hybridized Nucleic Acid Mixture with ≥1 Enzyme(s), containing at least one polymerase and/or ligase activity
Ligate into Vector
Introduce Ligated Nucleic Acids into cells
Propagate Cells Containing Ligated DNA
Optional: Initial Arraying of Library
Select Clones of Interest Using a Plurality of Screens, such as hybridization, complementation, binding activity or other expression-based Screen
Arraying of Nucleic Acids (e.g. of Pools, Subdivided or Fractionated Pools, Clones, Fragments, Sorted or Diluted DNA Fragments, etc. . .)
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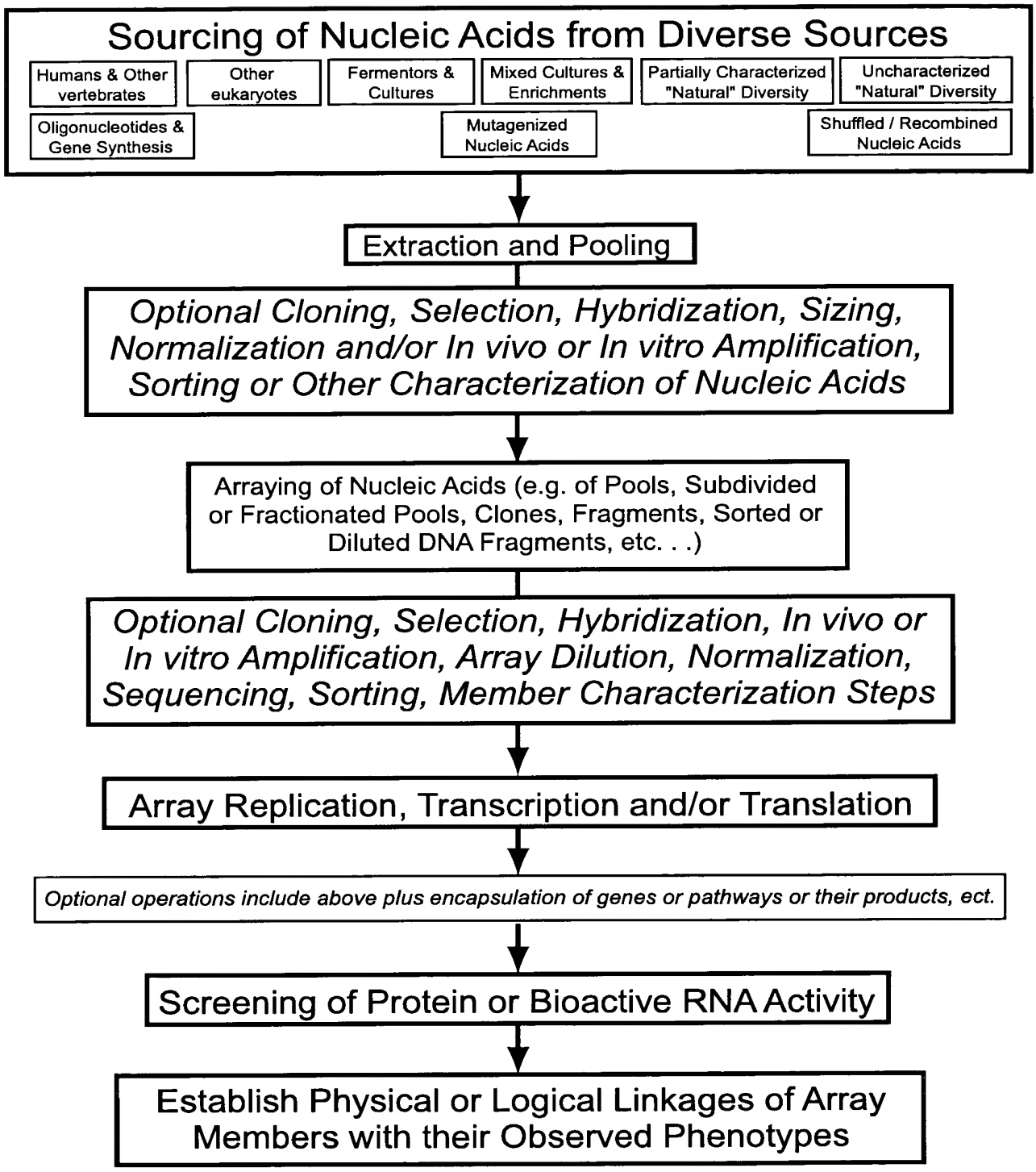
Screening of Protein or Bioactive RNA Activity
Establish Physical or Logical Linkages of Array Members with their Observed Phenotypes

Fig. 26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Fermentors & Cultures
Mixed Cultures & Enrichments
Partially Characterized "Natural" Diversity
Uncharacterized "Natural" Diversity
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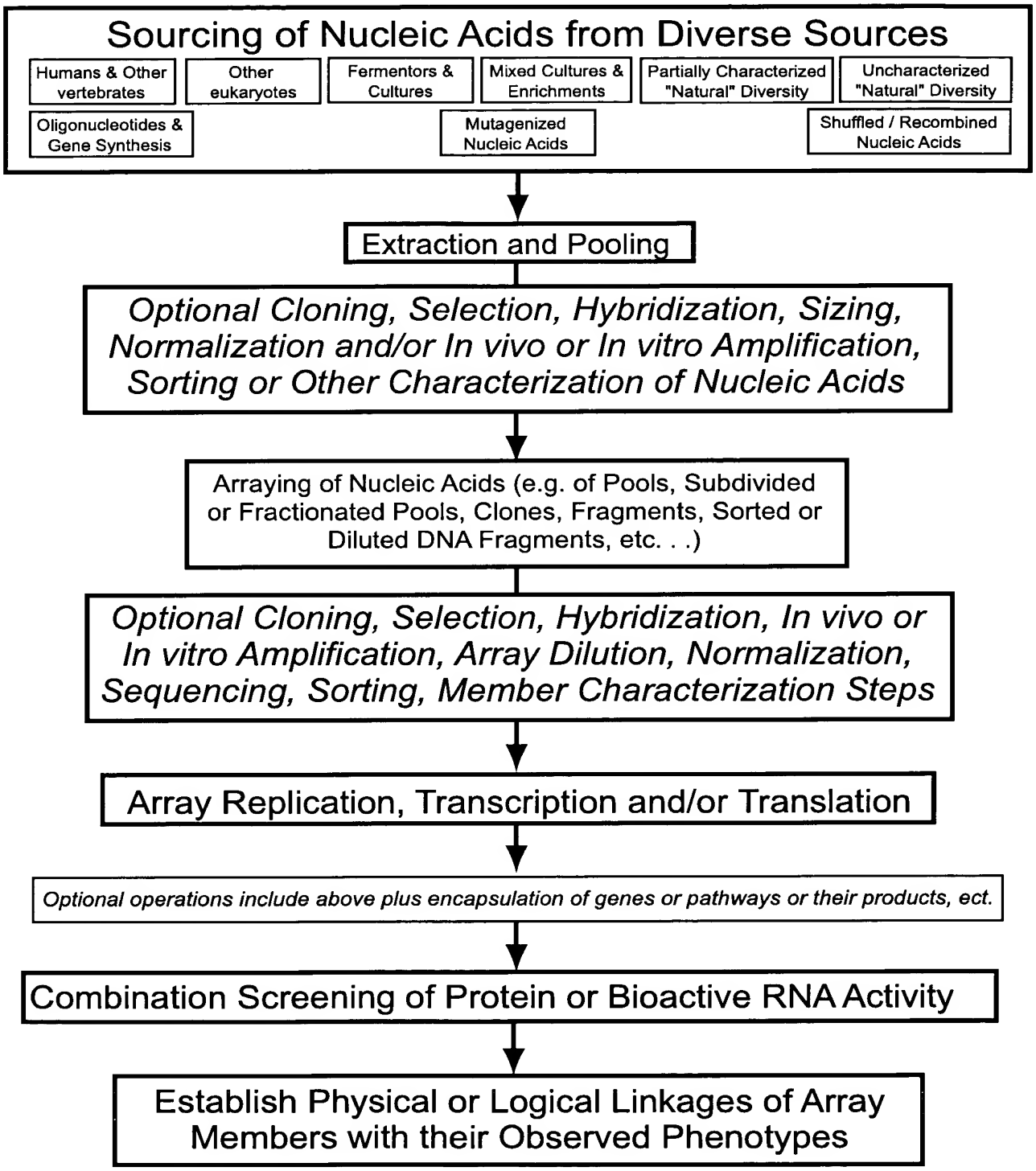
Array Replication, Transcription and/or Translation
Optional operations include above plus encapsulation of genes or pathways or their products, ect.
Screening of Protein or Bioactive RNA Activity
Establish Physical or Logical Linkages of Array Members with their Observed Phenotypes

Fig. 27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Uncharacterized "Natural" Diversity
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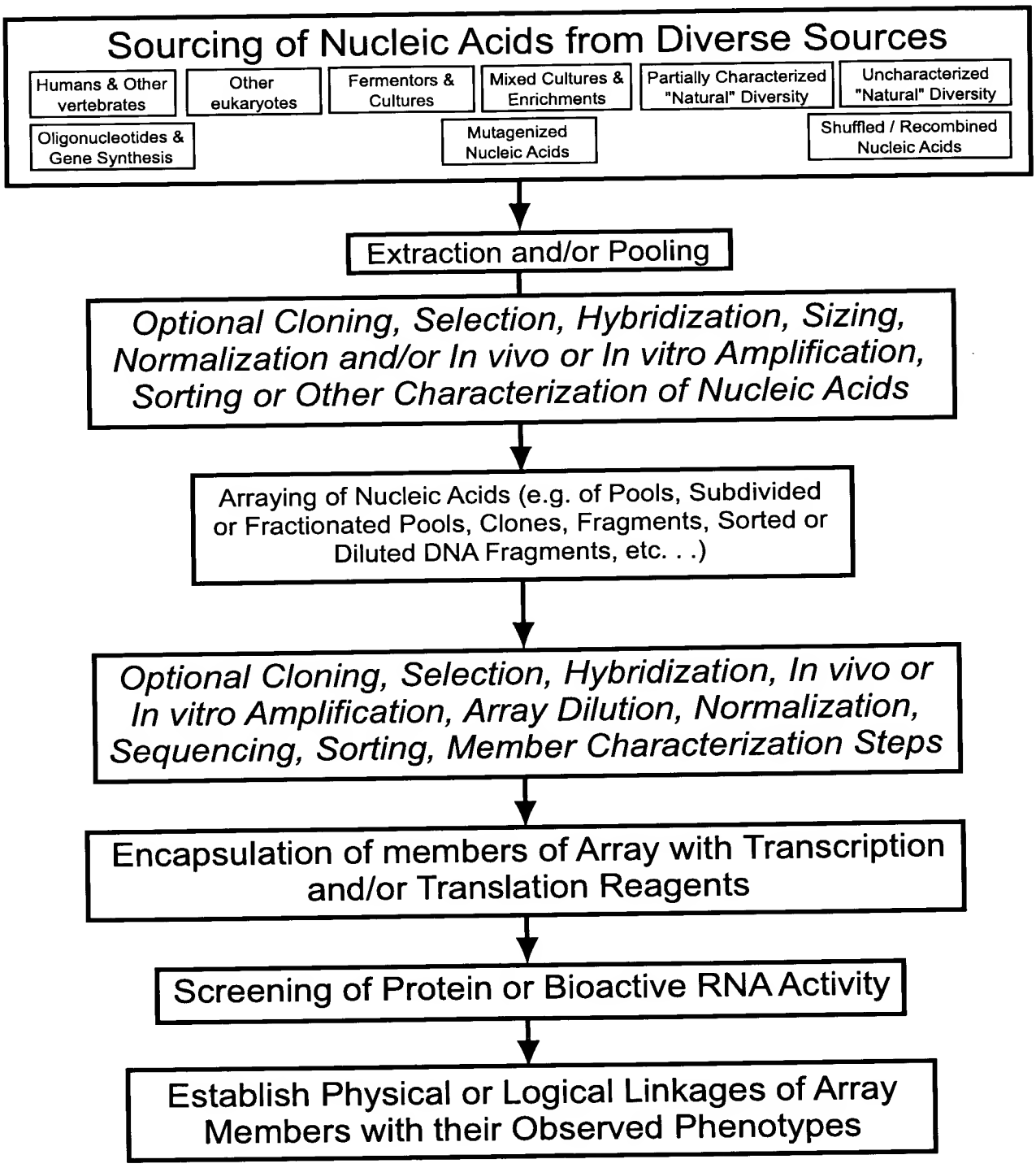
Optional Cloning, Selection, Hybridization, In vivo or In vitro Amplification, Array Dilution, Normalization, Sequencing, Sorting, Member Characterization Steps
Array Replication, Transcription and/or Translation
Optional operations include above plus encapsulation of genes or pathways or their products, ect.
Combination Screening of Protein or Bioactive RNA Activity
Establish Physical or Logical Linkages of Array Members with their Observed Phenotypes

Fig. 28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Uncharacterized "Natural" Diversity
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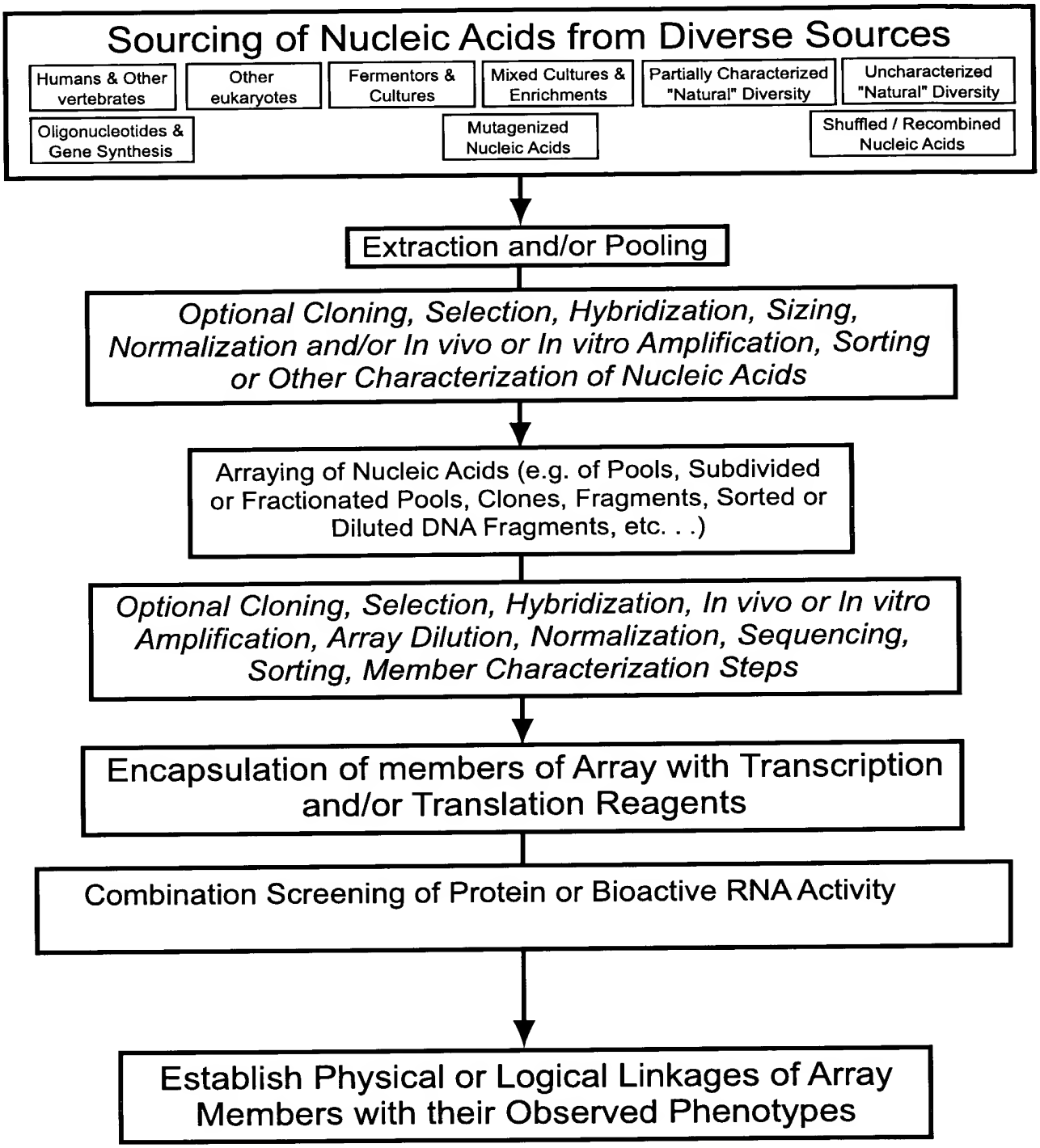
Arraying of Nucleic Acids (e.g. of Pools, Subdivided or Fractionated Pools, Clones, Fragments, Sorted or Diluted DNA Fragments, etc. . .)
Optional Cloning, Selection, Hybridization, In vivo or In vitro Amplification, Array Dilution, Normalization, Sequencing, Sorting, Member Characterization Steps
Encapsulation of members of Array with Transcription and/or Translation Reagents
Screening of Protein or Bioactive RNA Activity
Establish Physical or Logical Linkages of Array Members with their Observed Phenotypes

Fig. 29


Sourcing of Nucleic Acids from Diverse Sources
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Partially Characterized "Natural" Diversity
Uncharacterized "Natural" Diversity
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Mutagenized Nucleic Acids
Shuffled / Recombined Nucleic Acids
Extraction and/or Pooling
Optional Cloning, Selection, Hybridization, Sizing, Normalization and/or In vivo or In vitro Amplification, Sorting or Other Characterization of Nucleic Acids
Arraying of Nucleic Acids (e.g. of Pools, Subdivided or Fractionated Pools, Clones, Fragments, Sorted or Diluted DNA Fragments, etc. . .)
Optional Cloning, Selection, Hybridization, In vivo or In vitro Amplification, Array Dilution, Normalization, Sequencing, Sorting, Member Characterization Steps
Encapsulation of members of Array with Transcription and/or Translation Reagents
Combination Screening of Protein or Bioactive RNA Activity
Establish Physical or Logical Linkages of Array Members with their Observed Phenotypes

Fig. 30

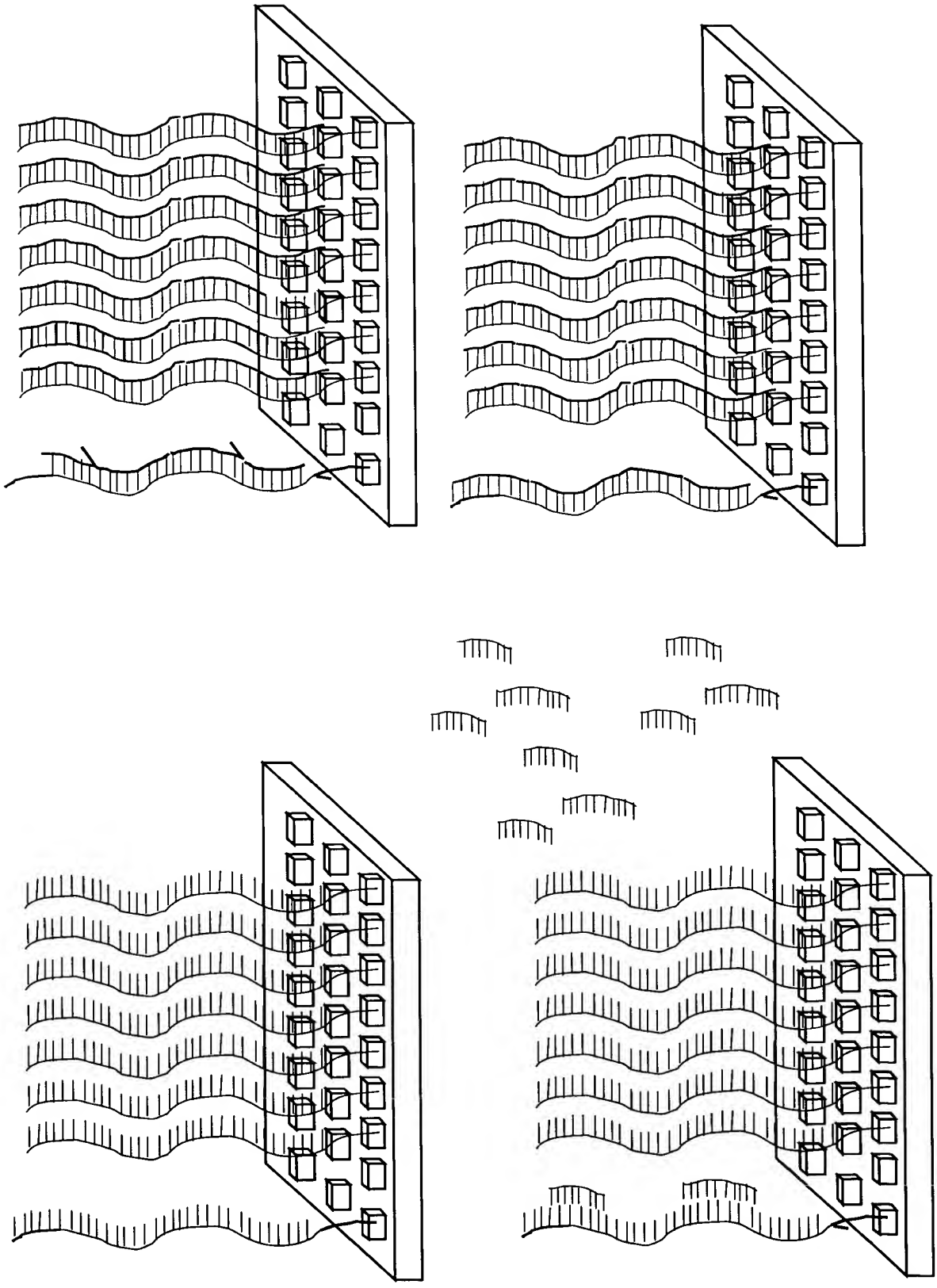

Fig. 31

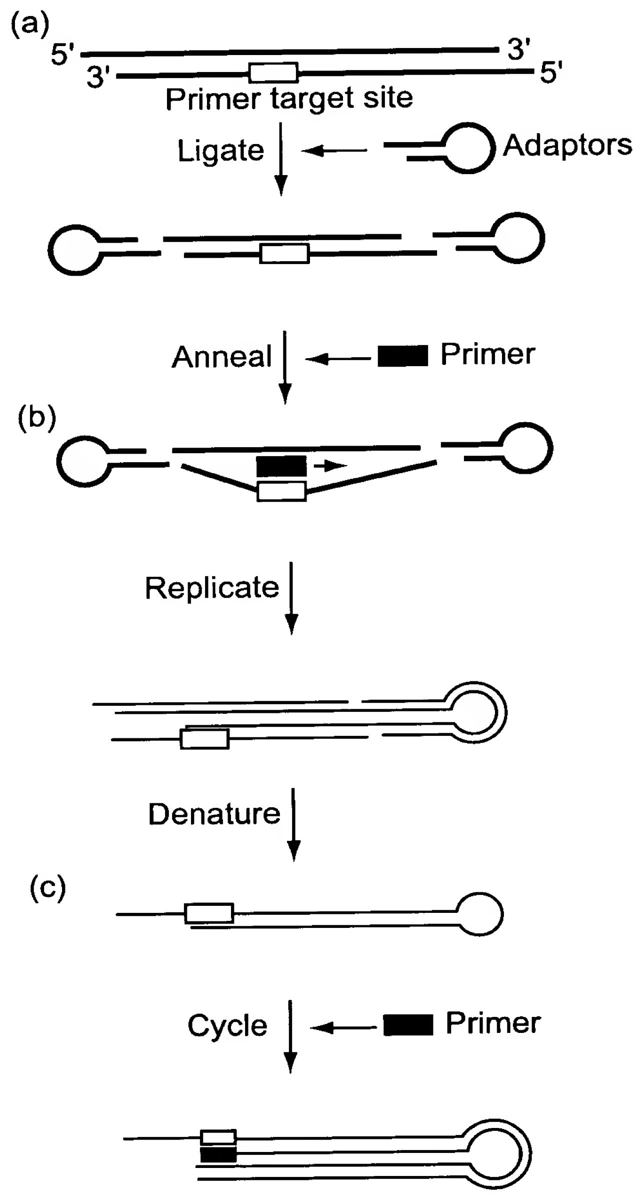

Fig. 32A

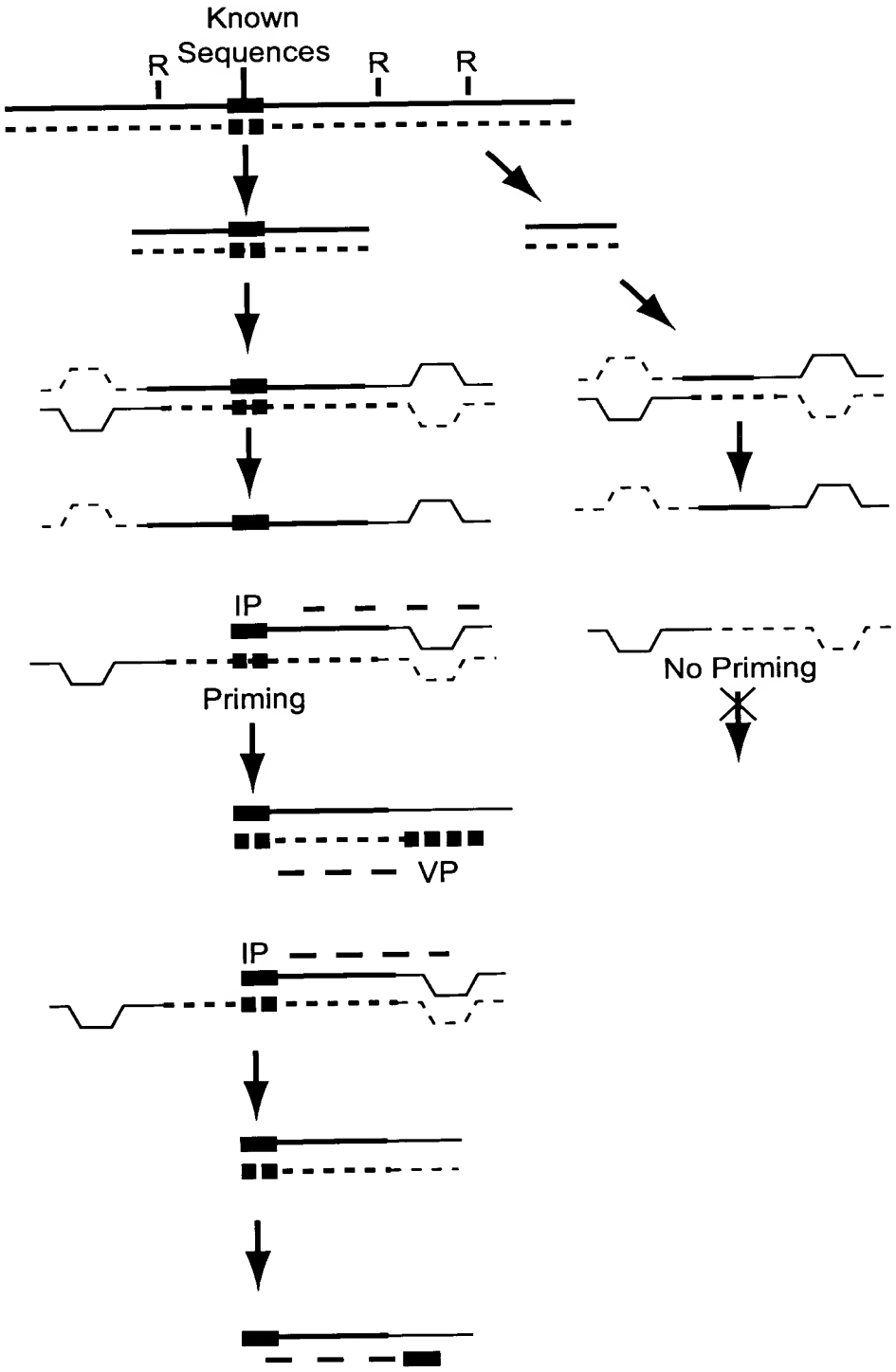

Fig. 32B

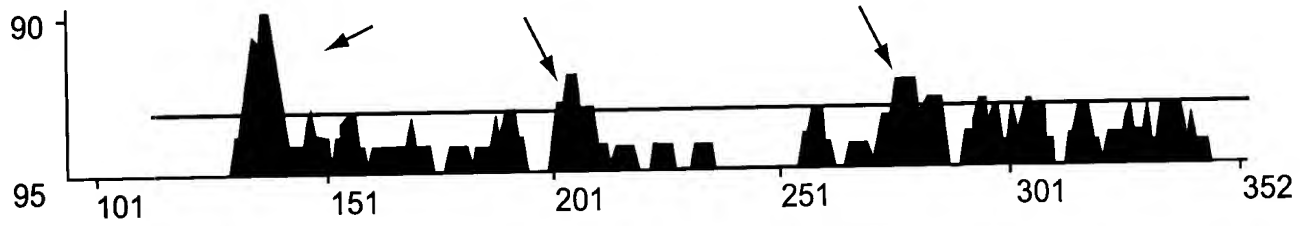

Fig. 33